SOCIAL REWARD IS INTACT IN BALB/C MICE, ATTENUATED BY POSTNATAL VALPROIC ACID TREATMENT, AND UNAFFECTED BY GSTM1 KNOCKOUT IN A NEURODEVELOPMENTAL ANIMAL MODEL OF AUTISM

by

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ABSTRACT OF THE DISSERTATION

Social reward is intact in BALB/c mice, attenuated by postnatal valproic acid treatment, and unaffected by GSTM1 knockout in a neurodevelopmental animal model of autism

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In a neurodevelopmental model of autism with age, genetic background and toxicant exposure as factors, three experiments assessed social reward in adolescent mice using an adapted social conditioned bedding preference task. Autism is a neurodevelopmental disorder behaviorally defined by restricted and repetitive activities and interests, core impairments in communication, and pervasive deficits in social interaction. Although no single causal agent has been identified, a plausible candidate may be early toxicant exposure in individuals with genetic vulnerability. The generation of reactive oxygen species may be a mechanism shared by toxicants such as valproic acid, which leads to autistic-like symptoms in both mice and humans. Thus, these experiments examined the effects of BALB/c strain, GSTM1 mice lacking an enzyme involved in oxidative stress, and BALB/c mice treated with postnatal valproic acid, on social conditioning in adolescent mice. Results revealed intact social conditioning in BALB/c mice and GSTM1 mice. However, postnatal valproic acid treatment led to social conditioning deficits and perseverative responding in adolescent BALB/c mice. Collectively, these results

corroborate previous studies suggesting that early postnatal toxicant exposure induces social deficits in adolescence. Use of the social conditioning paradigm allowed assessment of deficits in individual mice, reducing potential for experimental confounds during the test. Furthermore, these experiments lay the foundation for investigations of the effects of genotype-toxicant interaction on social conditioning or subsequent social behavior.

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TABLE OF CONTENTS

List of Figures

I. INTRODUCTION

Social interaction in typically developing mice

Social beings, from a diverse array of organisms from humans to the murine rodents, exist in the context of other individuals. Understanding that the brains of social organisms have evolved in a social context is critical in order to appreciate why deprivation of early social interaction can derail neurodevelopment and alter the trajectory of a lifetime of behavior (Hol, Van den Berg, van Ree and Spruijt, 1999). Even as social isolation engenders alterations in neural pathways that are problematic and persistent, adequate social interaction provides crucial experience to those pathways, setting the occasion for encountering future learning opportunities in the behavioral environment of an organism (Venderschuren, Stein, Wiegant and van Ree, 1995). Therefore, from a developmental perspective, it matters very much whether, and why, an infant tends to find social interaction to be "rewarding" or "aversive". Social interaction experienced as rewarding can facilitate opportunities for gains in communication acquisition (and encountering developmental behavioral cusps like generalized imitation; Rosales-Ruiz and Baer, 1997). If social contact is generally aversive, however, approaching conspecifics will not be as likely, limiting the opportunities for another person to function as a discriminative stimulus or mediate consequence delivery that normally maintains social interactions in the behavioral environment.

The social interactions that characterize the behavioral stream of adolescent rodents are different and more prevalent than those that follow sexual maturation. Adolescent animals engage in play fighting, social investigation, and general social proximity (Douglas, Varlinskaya, and Spear, 2004; Panksepp, Siviy and Normansell,

1984; Vanderschuren, Niesink, and van Ree, 1997), similar to the period highly important for peer interaction during human adolescence (Harris, 1995; Larson and Richards, 1991). These interactions function to organize communication systems between members of the species (Vanderschuren et al., 1997), providing crucial input to the neural pathways subserving communication, contextual learning and memory that deteriorate in the presence of social isolation (Ibi, Takuma, Koike, Mizoguchi, Tsuritani, Kuwahara, Kamei, Nagai, Yoneda, Nabeshima, and Yamad, 2007; Lu, Bao, Chen, Xia, Fan, Zhang, Pei, and Ma, 2003). But besides serving as communication or experience, an important function for the universal occurrence of increased social behavior during adolescence may be its property of reward (Calcagnetti and Schechter, 1992; Csikszentmihalyi, Larson, and Prescott, 1977; Panksepp and Lahvis, 2007; Panksepp, Jochman, Kim, Koy, Wilson, and Lahvis, 2007). Like the ultrasonic vocalization (USV) -eliciting effect of drug self-administration (Barker, Root, Ma, Jha, Megehee, Pawlak, and West, 2010), social experiences between adolescent peer mice are accompanied by positive affect-related USV's (Panksepp et al., 2007), even among the BALB/c strain often associated with low sociability (Sankoorikal, Kaercher, Boon, Lee, and Brodkin, 2005; see review by Brodkin, 2007). Furthermore, the contextual relationship between vocalizations and social interaction may suggest one of the interesting ways in which responses to social contextual stimuli provides information about future behavior: affective states can provide information about the kind of stimulus event in whose presence they occur, with positive affect related behavior predicting positively reinforced, but negative affect related calls predicting avoidance, behavior. Ultimately, adolescents learn much about the meaning of others' affective expression from peers.

The importance of social interaction during neurodevelopment

Fundamental to the age by environment by genetic model of autism is the idea that the timing of events (ranging from insults and damage to beneficial and important social experiences) critically contributes to their effects on neurodevelopment. Better characterization of biological contributions to variation in normal social interaction needs to take place before understanding its abnormal development. Consistent with its broad range of action in biological and behavioral functional relationships, social interaction plays diverse roles in rodent models of disorders. For example, social interaction can serve as a variable whose expression during adolescence is critically impaired by early treatment with toxicants (Yochum, Dowling, Reuhl, Wagner, and Ming, 2008) or one whose expression in adulthood is changed by problems like demyelination during the juvenile period (Makinodan, Yamauchi, Tatsumi, Okuda, Kiuchi, Sadamatsu, Wanaka, and Kishimoto, 2009). On the other hand, rich peer interactions adolescent mice can facilitate the richness of social interactions between adults (Vanderschuren et al, 1997; Branchi, 2009).

Meanwhile, in addition to its role as locus of important neurodevelopmental events, other areas of research have considered the ability of social interaction to support conditioning to be indicative of variable genetic contributions to social reward (Panksepp and Lahvis, 2007; Panksepp et al., 2007), or of genetic contributions to phenotypes of sociability (Crawley, Chen, Puri, Sullivan, Hill, Young, Nadler, Moy, Young, Constantino, and Todd, 2000; Moy, Nadler, Young, Perez, Holloway, Barbaro, Barbaro, West, Threadgill, Lauder, Magnuson, and Crawley, 2007). In these studies, as in the presently described experiments, interaction itself constitutes a conditioning experience

that can establish a preference for contextual stimuli paired with social interaction. Finally, social interaction or its absence can act as a contextual variable that modulates subsequent neuroimmunological or neuropharmacological events, as when aversive social interactions enhance virus specific immunological memory (Mays, Bailey, Hunzeker, Powell, Papenfuss, Karlsson, Padget, and Sheridan, 2010), act as a risk factor for drug reinstatement (Ribeiro, Aguilar, Lluch, Rodriguez-Arias and Minarro, 2009), or enhance vulnerability to disease (Bernberg, Andersson, and Gan, 2008McCabe, Gonzales, Zaias, Szeto, Kumar, Herron, and Schneiderman, 2002; Nation, Gonzales, Mendez, Zaias, Szeto, Brooks, Paredes, D'Angola, Schneiderman, and McCabe, 2008).

Genetic variables (Moy et al., 2007; Sankoorikal et al., 2005) and individual variation (in, for example, the distribution of receptors for the neurotransmitter and peptide oxytocin, highly expressed among members of species that form pair bonds such as prairie voles (Ross, Freeman, Spiegel, Ren, Terwilliger, and Young, 2009; Lim, Bielsky, and Young, 2009) and humans [Rodrigues, Saslow, Garcia, John, and Keltner, 2009]) contribute to the reinforcing nature of social interaction for a given organism. Even so, members of a species find social interaction with peers to be rewarding before sexual maturity (Panksepp et al. 2007; Pankepp, Siviy, and Normansell, 1984), suggesting a functional importance for the basis for the inherently rewarding property of social contact between conspecifics, or peer members of the same species (Panksepp, 1981; Van den Berg, Van Ree, Sprujit, Everts and Koolhaas, 1999; Meaney and Stewart, 1981). When the basis of social interaction is fundamentally disturbed, as occurs in the core social and communication deficits observed during a behavioral diagnosis of autism spectrum disorder, social interaction becomes the locus of crucial intervention. Following

diagnosis, a fight against time often ensues, when caregivers may mobilize resources to provide intensive early intervention aimed at ameliorating the very deficits at the crux of an autism diagnosis. But once autism is diagnosed (generally after the age of 3), the period critical for engaging neural plasticity mechanisms that subserve the pathways involved in neurodevelopment of social interaction may have passed.

Social interaction is fundamentally impaired in autism

Whereas the word *social* is derived from Latin words for "companion" and for "following", implying inherent and normal links between individuals, the word *autism* refers, at its root, to aloneness. According to the *Diagnostic and Statistical Manual of Mental Disorders-IV* (American Psychiatric Association, 2000), autism spectrum disorders are diagnosed by behavioral impairment in three areas including social interaction deficits, communication difficulties, and restricted and stereotyped activities and interests. Thus, while persons with autism are different from each other in myriad ways, impairments in social interaction are likely to be a constant, and to remain one of the most far-reaching features of the disorder. The extent to which a person with autism masters the social environment can have the greatest impact on his or her future quality of life, because it directly contributes to the expression and managing of impairments in the other behavioral categories, communication and interests (Koegel, Valdez-Menchaca, and Koegel, 1994). Since it is pragmatic to appreciate behavior in its most relevant context of occurrence, the next section addresses the disturbances in social behavior of children with autism, in a context of comparison to their typically developing peers.

An autism diagnosis is typically obtained around preschool age. However, increasing evidence indicates that at least in some cases, symptoms affecting social interaction such as impairments in joint attention are observable before an infant's first birthday. In a case study, Dawson, Osterling, Meltzoff, and Kuhl (2000) presented their observations on the social deficits that presented before a child was two years of age, and before the normal age of autism diagnosis. The impairments they observed between six months and one year included poor eye contact and deficits in imitation (failure to engage in imitative games and lack of vocal imitation responses), in a child who met criteria for diagnosis by shortly after one year of age. In fact, impairments in eye contact, shared affect, social orienting, and joint attention are behaviors that could be observed earlier than the typical preschool age of diagnosis (Dawson et al., 2000). Considerable progress has been made toward standardized, differential diagnostic criteria, and creation of tools that reliably and usefully identify treatment targets. Two such instruments are the widely-used Childhood Autism Rating scale (Schopler, Reichler, DeVellis, and Daly, 1980) and the Autism Diagnostic Interview-revised (Lord, Rutter, and LeCouteur, 1994), a standardized semistructured interview to facilitate differential diagnosis of pervasive developmental disorders. Like the *DSM-IV* (2000), the Autism Diagnostic Interview-revised, or ADI-R, assesses responses in the three content areas of social interaction, communication, and restricted, repetitive behaviors and interests. The ADI-R outlines the following symptoms in the domain of reciprocal social interaction: failure to use direct gaze, to engage in social smiling, to use range of facial expressions to regulate social interactions, to show interest in other children, respond to other children's approaches, show and direct attention; and a lack of seeking to share own enjoyment with others.

Within infant development, measures of interaction quantifying the quantity and depth of exchanges between infants and their caregivers and peers are particularly important, according to researchers including Mundy, Sigman, Ungerer, and Sherman (1986) who developed the Early Social Communication Scales to systematically elicit and assess joint communication, and Butterworth and Jarrett (1991) who assessed joint attention ability.

In the absence of large prospective studies to track the development of these behaviors and their relationship to autism, researchers have emphasized retrospective parental observations that have yielded much of the information on how autism presents in infancy (Osterling and Dawson, 1994). One of the earliest precursors to social conversation, joint attention refers to an infant's capacity to coordinate attention with a social partner with respect to a stimulus event. Joint attention has an important role in early acquisition of language (Bakeman and Adamson, 1984; Baldwin, 1995; Moreles, Mundy, Delgado, Yale, Messinger, Neal, and Schwartz, 2000; Mundy and Gomes, 1997). A measure similar operationalized by Scaife and Bruner (1975) is responding to joint attention, or following the gaze, head turn, and/or point, in response to a similar gesture by a social partner. Developing better capacity for early detection of emergent deficits or abnormal patterns in social development is especially important for human infants potentially at risk for autism, since the early and intensive treatment of core deficits in social communication is critically related to improved treatment outcome (Fenske, Zalenski, Krantz, and McClannahan, 1985; Howlin and Rutter, 1987; Jocelyn, Casiro, Beattie, Baw, and Kneisz, 1998; Lovaas, 1993; Ozonoff and Cathcart, 1998; Rosenwasser and Axelrod, 2002). Furthermore, corresponding to the temporal context of crucial developments taking place in human infants prior to the first year, the second postnatal

week is particularly important for normal affective emotional and social development in mice, as developmental events in the neural circuitry supporting these domains are especially complex and dynamic during this time (Judson, Bergman, Campbell, Eagleson, and Levitt, 2009).

Peer interventions for students with autism: A critical locus of early intervention

The deficits in social interaction that have been described so far in children with autism present during childhood as specific impairments in the social context of interaction with same age peers. With respect to other children of similar ages, children with autism are less able than their typically developing peers to respond to other children, even though they responded readily to initiations from adults (Jackson, Fein, Wolf, Jones, Hauck, Waterhouse, and Feinstein, 2003). In terms of initiations, children with autism showed similar initiations to adults but lower rates of initiations to peers (Hauck, Fein, Waterhouse, and Feinstein, 1995). Supporting the locus of peer interactions as critical for socially meaningful intervention, evidence reveals peer-mediated strategies as among the most useful in increasing social interaction between a child with autism and a typically developing peer (Weiss and Harris, 2001). Peer mediated strategies generally include three techniques. The first technique is proximity (Odom and Strain, 1984), or the spatial closeness between children that is typical of inclusive educational environments (Johnson and Johnson, 1984; Roeyers, 1996). When multiple peer partners are involved, this training technique can result in treatment gains for children with autism; these gains can then generalize to multiple peers (Roeyers, 1996). However, mere proximity as a result of classroom placement with typically developing peers is often not sufficient to

produce substantial improvements in interaction for students with disabilities (Gresham, 1984; Snyder, Apolloni, and Cook, 1977). Other peer mediated strategies include teaching or telling peers to prompt, reinforce, and initiate interactions with children with autism (Odom and Strain, 1994). Inherent in the current study is an attempt toward understanding, in an animal model of autism, the neurobiological basis for the effectiveness of peer-mediated interventions. As will be later described, our social contextual conditioning task constitutes predictable experience with peers that are nonlittermate conspecifics. In that these predictable social encounters provide occasions to experience proximity to novel peers, we are developing an animal model not entirely unlike some effective social autism interventions (Weiss and Harris, 2001) in the autism treatment literature. Like those of humans, adolescent peer-directed social interactions between rodents are prerequisites for receptive and expressive communication (Meaney and Stewart, 1981; Takahashi, 1986).

Valproic acid is teratogenic to the developing organism

In addition to the previously described disturbances in behavior present in autism, other features include evidence of concurrent clinical (Ming, Brimacombe, Zimmerman-Bier, Chaaban, and Wagner, 2008), neurochemical (Nelson, Grether, Croen, Dambrosia, Dickens, Jelliffe, Hansen, and Phillips, 2001) or immunological dysfunction. Among these markers, the high rate of proinflammatory cytokine production observed in individuals with autism (Li, Chauhan, Sheikh, Patil, Chauhan, Li, Ji, Brown and Malik, 2009; Molloy, Morrow, Meinzen-Derr, Schleifer, Dienger, Manning-Courtney, Altaye, and Wills-Karp, 2006) makes it imperative to understand the exquisite sensitivity of the

immune system to exposure to environmental toxicants, such as sodium valproate (valproic acid, or VPA). Studies performed with genetically vulnerable mice confirm that the dysfunctional changes in the immune system exposed during gestation to environmental toxicants include higher generation of reactive oxygen species (ROS) and greater production of proinflammatory cytokines in early postnatal life (Blossom, Doss, Hennings, Jernigan, Melnyk, and James, 2008). Contact with toxicants can result in sex-specific changes in the behavior of gestation-exposed juveniles (Blossom et al., 2008; Schneider, Roman, Basta-Kauim, Kubera, Budziszewska, Schneider, and Przewlocki, 2008). The links between social challenges experienced by individuals with autism, and exposure to potentially involved environmental toxicants, have been more explicitly investigated using the neurodevelopmental teratogen VPA.

Used as an antiepieptic drug to modulate neurophysiology, a clinically relevant dose of sodium valproate controls seizure activity for epilepsy-affected adults and children (Davis, Peters, and McTavish, 1994; Loscher, 2002). Unfortunately, its usefulness is compromised by teratogenicity during fetal development (Ornoy, 2009) and its association with increased oxidative stress (Chang and Abbott, 2006; Michoulas, Tong, Teng, Chang, Abbott, and Farrell, 2006; Naziroglu, 2009). Environmental exposure to valproic acid during the embryonic period or early postnatal development has especially adverse consequences for the developing organism, including the increased likelihood of neural tube deficits or the occurrence of “fetal valproate syndrome”, which produces developmental problems and communication disturbances resembling those observed in the clinical diagnosis of autism spectrum disorders. In order to better understand the relationship between exposure to environmental teratogens and

neurodevelopmental disorders such as ASD's, animal models involving valproic acid exposure have been useful to study the biomarkers associated with autism such as disturbed neurochemical profiles (Narita, Kato, Tazoe, Miyazaki, Narita, and Okado, 2003), including hyperserotonemia (Anderson, Horne, Chatterjee, and Cohen, 1990).

Valproic acid is used as an autism-like symptom inducing factor in some genetic by environmental by age models of autism in large part due to its tendency to engender, in both humans and rodents, neurodevelopmental and social behavioral abnormalities structurally similar to those involved in autism pathogenesis. In addition to altering the neurobehavioral development of young mice, early exposure to valproic acid reduces Purkinje cell number and cerebellar cell volume in rodents, similar to the histopathological abnormalities observed in patients with autism (Ingram, Stodgell, Hyman, Figlewicz, Weitkamp, and Rodier, 2000; Rodier and Hyman, 1998). Metabolism-dependent oxidative stress is a mechanism by which valproic acid results in cytotoxicity (Jurima-Romet, Abbot, Tang, and Whitehouse, 1996; Simon, Moog, and Obert, 1994). Supporting the idea that the pathogenesis of autism involves increased apoptosis and inflammation (Vargas, Nascimbe, Krishnan, Zimmerman, and Pardo, 2005; Li et al., 2009), capthepsin D, which normally regulates apoptosis, is increased in the cerebellum and hippocampal granule and pyramidal cells of patients with autism (Sheikh, Li, Wen, Tauqeer, Brown, and Malik, 2010). Importantly, the generation of reactive oxygen species appears to be a mechanism shared by toxicants that have been associated with autism or autistic-like symptoms in both mice (Wagner, Reuhl, Cheh, McRae, and Halladay, 2006; Yochum et al., 2008; Cheh, Halladay, Yochum, Reuhl, Polunas, Ming, and Wagner, 2010) and humans (Kern and Jones, 2006; Ming, Johnson, Stenroos, Mars,

Lambert and Buyske, 2010; Moore, Turnpenny, Quinn, Glover, Lloyd, Montgomery, and Dean, 2000). Evidence suggests that persons genetically vulnerable to oxidative stress are at greater risk for the deleterious effects of environmental toxicants (Chahine, Baccarelli, Litonjua, Wright, Suh, Gold, Sparrow, Vokunas, and Schwartz, 2007; Schwartz, Litonjua, Suh, Verrier, Zanobetti, Syring, Nearing, Verrier, Stone, MacCullum, Speizer, and Gold, 2005). One relevant locus of action is the enzyme glutathione-S-transferase M1 (GSTM1), which detoxifies xenobiotics via glutathione conjugation (Armstrong, 1997). A deficiency in the expression of this enzyme has been associated with autism (Buyske, Williams, Mars, Stenroos, Ming, Wang, Sreenath, Fractura, Reddy, Lambert, and Johnson, 2006). As the major intracellular (endogenous) antioxidant in organisms, the enzyme glutathione (GSH) is a defender against oxidative stress (Lei, Cheng and McClung, 2007). Without enough enzymatic activity to break them down, accumulated reactive oxygen species (ROS) can generate oxidative stress and result in cellular damage, ultimately contributing to cell death and disease (Valko, Rhodes, Moncol, Izakovik, and Mazur, 2006). Especially unfortunate, social interaction that is aversive or stressful may exacerbate dysfunction in the dynamic pathways concerned with both immune function and glutathione-related oxidative stress (Goncalves, Dafre, Carobrez, and Gasparotto, 2008; DiRosa, Gangemi, Cristani, Fenga, Saitta, Abenavoli, Imbesi, Speciale, Minciullo, Spatari, Abbate, Saija, and Cimino, 2009).

Behavioral and neurophysiological disturbances reminiscent of those in autism spectrum disorders can be consequent to toxicant exposure at critical periods of neurodevelopment. For example, humans who have been prenatally exposed to valproic acid show behavioral and neuroanatomical abnormalities similar to those observed in

autism (Ardinger, Atkin, Blackston, Elsas, Clarren, Livingstone, Flannery, Pellock, Harrod, and Lammer et al., 1988; Moore et al., 2000; Williams, King, Cunningham, Stephan, Bronwyn, and Hersh, 2001). Consistent with the social impairments observed in human fetal valproate syndrome (Williams et al., 2001), deficits in social behavior are induced in rodent models of autism using prenatal (Schneider and Przewlocki, 2005; Schneider, Turczak, and Przewlocki, 2006) or postnatal (Wagner et al., 2006; Yochum et al., 2008) exposure to valproic acid. In this model of autism, administration of valproic acid on PND14 (postnatal day 14) roughly corresponds to the particularly vulnerable third trimester of human development (Rice and Barone, 2000). At this time, hippocampal and cerebellar granule cells are undergoing migration and differentiation (Bachevalier and Beauregard, 1993; Rice and Barone, 2000; Voorhees, 1986). Ultimately, this research joins a body of work documenting alterations in the future social behavior of individuals exposed during perinatal or early postnatal development to chemical compounds (e.g., Hill, Fontana, McCloskey and Commissaris, 1992; Bekkedal, Panksepp and Rossi, 1998).

VPA treatment in rodent models of autism

In the light of evidence for VPA-induced neuropathological damage and behavioral deficits reminiscent of features of autism, several recent studies (Schneider and Przewlocki, 2005; Schneider et al., 2006; Wagner et al., 2006; Yochum et al., 2008) have employed prenatal or postnatal exposure to valproic acid in rodent models of autism, positing that age and toxicant exposure may critically interact with neurodevelopment to produce behavioral impairments in social interaction. Observing

that exposure during day 20-24 of gestation to teratogens leads to autism in human children (Miller and Stromland, 1993), Schneider and Przewlocki (2005) described a rat model of autism in which valproic acid is administered during prenatal development to correspond with human gestation events. In addition to changes in postnatal motor development and olfactory discrimination induced by E12.5 treatment with valproic acid, Schneider and Przewlocki (2005) recorded alterations in the play and social behavior of adolescent (30-35 day old) rats. Even as this model does not account for the vast difference between the complexities of both normal and aberrant social behavior of human individuals compared to members of rodent species, an element of face validity is present in that valproic acid disrupted olfactory guided behavior in infant rats, play behavior of adolescents, and social behavior of adults exposed in utero (2005). These observations are consistent with the hypothesis that events capable of disrupting early affiliative behavior compromise the future ability to understand and participate in intraspecies communication (Vanderschuren et al., 1997).

Subsequent to the demonstration of valproic acid induced alterations in motor skills and behavioral tasks during rat development, Schneider, Turczak and Przewlocki (2006) observed reversal of these behavioral alterations via a postnatal period of environmental enrichment. This effect is reminiscent of the way in which behavioral symptoms of autism spectrum disorders can be ameliorated with intensive early behavioral intervention (Fenske et al., 1985; Howlin and Rutter, 1987; Lovaas, 1993; Ozonoff and Cathcart, 1998; Rosenwasser and Axelrod, 2002), and suggestive of future research targets to understand possible biomarkers related to the alteration and improvement of social behavior across the lifespan of individuals with autism. Adding an

element of clinical validity to the valproic acid model of autism, Wagner et al. (2006) proposed that behavioral disruptions produced by interactions between age, genetic susceptibility, and environment be operationally defined in terms of developmental regressions, intrusions and deficits (see below). Furthermore, by extending the VPA model to mice, Wagner et al. (2006) opened the door to assessing social interaction deficits produced by valproic acid in combination with genetic polymorphisms known to be linked with autism in humans.

Age by toxicant exposure by genetic susceptibility model of autism

Wagner et al. (2006) and Yochum et al. (2008) documented that early postnatal treatment of mice with valproic acid impairs the development of cognitive, social, and motor skills. In this model, the neurobehavioral deficits induced by early valproic acid exposure are classified as retardations (i.e., a behavioral skill that matured later in the treated mice as compared to controls), regressions (i.e., a behavioral skill that matured on schedule but was then lost subsequent to valproic acid exposure), or intrusions (i.e., the valproic acid exposure induced behaviors of abberant intensity or frequency that overshadowed the normal maturation of behavioral skills) (Wagner et al., 2006). BALB/c mice were treated with 400 mg/kg of valproic acid on P14, resulting in significant impairment of cognitive performance on the hippocampal-dependent water maze task. In this task, mice put into an opaque water-filled tub must locate a hidden platform. Escape latency, or the delay between entering the tub and locating the platform, was prolonged on each of four days of testing in mice given 400 mg/kg of valproic acid on P14, indicating behavioral retardation. Other mice were given 200 mg/kg of valproic acid on

P26 after demonstrating the mature skill of escape to the hidden platform, resulting in regression of the learned cognitive skill. Mice treated with either 400 mg/kg or 200 mg/kg doses of valproic acid showed regression of mid-air righting (a motor skill). Examining the effects of postnatal VPA administration on behaviors in the social domain, Yochum et al. (2008) found that between P30-P40, treated adolescents engaged in reduced ano-genital sniffs, allogrooming, and crawl-under/over behaviors compared to control mice. Corresponding to impaired neurodevelopment of cognitive and social skills were observations of significant increases in hippocampal cells staining for apoptosis 24 hr after injections of 400 mg/kg valproic acid, compared to saline-injected controls (Yochum et al. 2008).

Collectively, individuals with autism appear to share genetic susceptibility to oxidative stress (Chauhan and Chauhan, 2006; James, Melnyk, Jernigan, Cleves, Halsted, Wong, Cutler, Bock, Boris, Bradstreet, Baker and Gaylor, 2006) that can be conferred by genetic polymorphisms related to the glutatione-S-transferaseM1 (GSMT1) gene, which codes for a normally ubiquitous enzyme involved in oxidative stress regulation (Castro-Giner, Kunzli, Jacquemin, Forsberg, Sunyer, Jarvis, Briggs, Vienneau, Norback, Gonzales, Guerra, Janson, Anto, Wjst, Heinrich, Estivill, and Kogevinas, 2009; Yang, Parsons, Chi, Malakauskas, and Thu, 2009). In order to understand whether developmental teratogen exposure is potentiated in genetically susceptible mice, Yochum, Bhattacharya, Patti, Mirochnitchenko, and Wagner (2010) administered postnatal valproic acid treatment during postnatal (P14) development in mice with deletion of GSTM1. VPA treatment induced social behavior deficits accompanied by

significant increase of apoptosis in the cerebellum and hippocampus for all groups except wild-type females.

Overview of experiments and hypotheses tested

Foremost, the purpose of this series of experiments was to adapt the social contextual conditioning task first described by Panksepp and Lahvis (2007; Panksepp et al., 2007) for use in neurodevelopmental models of autism that consider combinatory effects of genetic susceptibility and environmental insults (Wagner et al., 2006; Yochum et al., 2008; Cheh et al., 2010). The utility of such an adaptation can provide an assay in which vulnerability to social deficits is observed in the absence of a peer partner. The social conditioning task is amenable for use in early adolescence during the period of time when species-typical social reward is not yet dependent on sexual maturity. This is a necessary development in animal models of autism that purport to understand the sensitivity of affective social deficits in autism affecting peer interactions. To these ends, Experiment 1a characterized the minimal conditions under which social contact between non-littermate peer adolescent mice from the BALB/c and C57BL/6 strains reliably result in conditioning. It was expected that, based on the results of previous studies (Panksepp and Lahvis, 2007; Panksepp et al., 2007) and pilot work in this laboratory, social contextual conditioning would, at least to some degree, increase approach to paired bedding in both strains.

The following experiments all used variants of the social contextual conditioning paradigm. In all experiments described herein, each animal's baseline approach to novel bedding materials serves as its own control, against which a subsequent 'preference'

could be conditioned. That is, one of two novel bedding materials was always used as the contextual stimulus paired with unconditioned reward, and the bedding first identified as 'nonpreferred' was always used as the specific contextual stimulus. As a positive control experiment for both BALB/c and C57BL/6 mice (Experiment 1b), highly palatable food was used as the unconditioned reward paired with nonpreferred bedding. It was expected that for BALB/c and C57BL/6, food conditioning would be equally if not more successful in conditioning approach to paired bedding.

Experiment 2 examined whether the neurodevelopment of social reward is altered by deletion of the GSTM1 gene, by assessing the effects of social contextual conditioning and food conditioning on approach to paired bedding for GSTM1 knockout and GSTM1 wild-type mice. Given previous documentation of association between glutathione-S-transferaseM1 polymorphisms and autism (Buyske et al., 2006), as well as the enhanced detrimental effects of valproic acid in GSTM1 knockout mice (Yochum et al. 2010), social deficits were expected in knockout compared to wild-type mice in social contextual conditioning.

The final experiment (3) sought to characterize the effects postnatal treatment with valproic acid on social contextual conditioning in BALB/c mice. The 400 mg/kg dose and timepoint at administration was selected as maximally effective in inducing social deficits and apoptosis in cerebellum and hippocampal regions (Yochum et al. 2008) during a period of time corresponding to the third trimester in human development (Rice and Barone, 2000). Although the social contextual conditioning paradigm had not been previously employed in the valproic acid model of autism, the previous findings from this laboratory and others showing social interaction deficits in VPA treated pups

predicted that social contextual conditioning would be impaired in adolescent BALB/c mice given postnatal VPA treatment.

If successful, these studies could replicate findings suggesting that postnatal exposure to valproic acid may disrupt social reward processes (Yochum et al., 2008). Using the social contextual conditioning paradigm to enhance the ability to assess social deficits in animal models of autism (Cheh, Millonig, Roselli, Ming, Jacobsen, Karndar, and Wagner, 2006, Yochum et al., 2008), may improve our understanding of how environmental toxicants may affect individuals with inherited vulnerability to oxidative stress (Yochum et al., 2010). In summary, it was hypothesized that for BALB/c and C57BL/6 mice, social contextual conditioning will result in increased approach of social contact-paired beddings, but not those paired with isolation (Experiment 1); and that either valproic acid (Experiment 3) or deletion of the GSTM1 gene (Experiment 2) could result in attenuated social contextual conditioning in BALB/c and GSTM1 -/- mice, respectively.

II. METHOD

Subjects and setting

Breeding colonies of GSTM1 +/+ and GSTM1 -/- on a C57B16J x 129 SvEv background, as well as C57BL/6 and BALB/c colonies, were established. All animals were maintained in a Psychology Department colony room at Rutgers University under standard vivarium conditions, with *ad libitum* food and water and a 12:12 hour light:dark cycle. All procedures were approved by the Animal Care committee and were in accordance with AAALAC guidelines. Sibling mice of the same confirmed genotype were housed together in pan cages (3 females and 1 male per cage) and allowed to breed. Mice were monitored and the day pups were born was recorded as postnatal day 0 (P0). Mice were weaned on P21 into same sex housing with up to three littermates and housed with free access to food and water.

All pharmacological manipulations and behavioral training and testing took place during the dark phase of the 12 hr light/dark cycle. Behavioral testing was conducted under red lighting in an otherwise dark room. Prior to all behavioral testing, animals were transported approximately 10 meters from the colony room to the testing room in their home cage on a cart.

A total of 105 mice participated as subjects in the present study. BALB/c subjects totaled 40 mice, including 19 females and 21 males, from a total of 12 litters. C57BL/6 subjects were 29 mice, including 14 females and 15 males, from a total of 9 litters. GSMT1 knockout mice totaled 19 mice (12 females and 7 males) from 6 litters, and GSTM1 wild-type mice were 17 animals (11 females and 10 males) from 4 litters. All

subjects were 21 days old at the start of the study. Prior to the conditioning phase of the experiment, all subjects were housed in laboratory standard beta chip bedding.

Procedures and apparatus

Overview of baseline, contextual conditioning and extinction testing

For all subjects, baseline assessment took place on P22, the conditioning phase of the experiment took place between P22-29, and the testing phase took place on P30-P31. Mice in Experiment 1 (both a and b), Experiment 2, and Experiment 3 participated in conditioning as described below. Pretest (baseline) and post-test (testing after conditioning) took place in the same apparatus and testing room. Data collection was conducted by experienced observers trained to mastery criterion on an observation code. Inter-observer agreement was computed regularly by two observers simultaneously recording the behavior of one subject. The primary behavioral measures were duration and frequency of movement bouts (in which a subject contacted bedding in a given location of the testing arena). Onset and offset of each bout was scored by an observer using a stopwatch. All testing was conducted by observers blind to subject's sex, genotype, previous conditioning history, and toxicant condition.

<u>Phase 1: Pretest on P21</u>

On the day of weaning (P21), a pretest determined the baseline duration of time spent in each of two bedding materials (pine chip and corn cob) that were novel to the subject. The pretest (10 min) took place in a covered behavioral chamber made of transparent polypropylene (41 x 19.5 x 23.5 cm), the floor of one side strewn with pine

bedding and the other with corn cob bedding. The position placement of specific bedding type within the chamber was counterbalanced across subjects during baseline. No physical barrier separated entry to the two sides of the chamber, allowing animals to move freely during the session. Onset of the pretest session occurred when the mouse was placed into the center of the chamber and offset of the pretest session occurred when 10 min had passed, when the experimenter removed the mouse from the observation chamber. The number of entries into each side (crossovers), the individual and average duration of bedding visits, and the total duration of time spent in each of the two sides (total duration) was recorded by a trained observer. The chamber was positioned within an activity monitor (Opto-Varimex Minor, Columbus Instruments, Columbus, OH) so that locomotor activity could be assessed. The motor activity chamber consisted of a Plexiglas box measuring 42 x 22 x 14 cm. Six infrared sensors approximately 7 cm apart and 2.5 cm above the floor were used to count the number of horizontal movements made by an individual mouse during the test. One crossing of a beam represented a unit of horizontal movement. The chamber was cleaned between all tests and new clean bedding was used for each subject.

Phase 2: Social contextual conditioning (P22-P29)

Social contextual conditioning began on the day after weaning (P22). Social contextual conditioning consisted of regular alternation between social housing in one bedding, and isolate housing in a different bedding. Either pine or cob bedding, both novel before baseline assessment, was labeled as ‘nonpreferred’ upon identifying which bedding was least approached during the two choice bedding baseline assessment. In

Experiments 1a, 2, and 3, this 'nonpreferred' bedding was paired with either social contact during paired trials. Social contact was defined as a regular period, beginning at the same time each day, of housing together with a non-littermate peer mouse matched for age, sex, strain, toxicant condition, and weight. Because both Panksepp and Lahvis (2007) and pilot work in our laboratory found that a period of social deprivation is a critical prerequisite for the expression of increased social preference after training, the sequence of alternations during conditioning always began with a paired trial and ended with an unpaired trial. Finally, the number and duration of trials was chosen based on pilot work in this laboratory that determined the minimum conditions to produce conditioned approach to social paired stimuli. For social contextual conditioning, BALB/c mice were always conditioned using 8 alternating trials lasting 24 hr each, and C57BL/6, GSTM1 knockout and GSTM1 wildtype mice were always conditioned using 4 alternating trials lasting 48 hr each. Thus, social contextual conditioning always lasted 8 days, began with a paired trial, and ended with an unpaired trial (e.g., a period of time in isolate housing).

Positive control experiment protocol

Like social contextual conditioning, food contextual conditioning took place on P22 and ended on P29. A pretest session took place on P21 to assess baseline preferences for the two beddings used during training as described previously. Beginning on P22, non food-deprived mice were exposed to 8 days of training trials (alternating 24 hr periods paired or unpaired with palatable food). During paired trials, subjects were housed for 24 hr in the bedding identified as 'nonpreferred' or least approached during baseline. On

paired trial onset, sweet cereal (7 pieces) was arranged on top of the bedding. On paired trial offset, remaining cereal was removed and counted. During unpaired trials, subjects were housed for 24 hr in the other bedding absent of any experimenter-presented stimuli. Thus, food contextual conditioning always lasted 8 days, began with a paired trial, and ended with an unpaired trial (e.g., a period of time in isolate housing in the absence of sweet cereal).

Phase 3: Extinction testing

For subjects in all three experiments, extinction testing began 24 hr after the last housing alternation, and consisted of a daily (10 min) test on 3 consecutive days. Thus, extinction testing took place on P30-P32. During the 3 days of the extinction phase, each subject was housed in standard beta chip bedding in isolation and transported in their cage to the testing room within 30 minutes prior to the test. The subject was placed into a testing arena under conditions similar to the pretest assessment; the floor was covered with bedding materials used during training. The location of each of the two bedding types on the floor was randomly determined on the first day of testing and counterbalanced each day thereafter for each animal. All testing was conducted by observers blind to subject's sex, genotype, previous conditioning history, and toxicant condition.

Postnatal treatment with sodium valproate (valproic acid, or VPA)

BALB/c mice were randomly assigned to receive a subcutaneous injection of either valproic acid (400 mg/kg) dissolved in physiological saline or vehicle only on

postnatal day 14 (P14). Subjects were weighed and injected at the start of the dark cycle and allowed to recover for 24 hours before body weight was monitored again. The body weight of BALB/c saline-injected subjects did not statistically differ from that of toxicant-injected subjects on P14, P15, or P16 ($p > 0.05$, data not shown). VPA-treated subjects remained in standard housing with their dams for the remainder of the preweaning period, were weaned on P21, and given a baseline pretest followed by social contextual conditioning as described above.

Experiment 1: Effects of strain on social or food contextual conditioning in BALB/c and C57BL/6 mice

Experiment 1a (N=15) examined the effects of social contextual conditioning (SCC) in BALB/c and C57BL/6 mice. The BALB/c social contextual conditioning group (BALB-Social) included 9 mice representing 3 litters, and the C57BL/6 social contextual conditioning group (C57-Social) included 6 mice from 2 litters. The positive control Experiment 1b (N=20) examined the effects of food contextual conditioning (FCC) in BALB/c (n=9) and C57BL/6 (n=11) mice. Of the BALB/c mice receiving SCC, 3 females and 6 males from a total of 3 litters participated, while BALB/c mice receiving FCC included 5 females and 4 males from 5 litters. Likewise, of the C57BL/6 mice receiving SCC, 3 females and 3 males from 3 litters participated, while C57BL/6 mice receiving FCC included 3 females and 8 males from 5 litters.

Experiment 2: Effects of GSTM1 genotype on social or food contextual conditioning

Experiment 2 (N=36) examined the contributions of genotype and conditioning type on approach to paired bedding. GSTM1 knockout mice included 19 mice, with 10 subjects in the social contextual conditioning experiment (KO-Social, n=10) and 9 subjects in the food contextual conditioning experiment (KO-Food, n=9). Of these knockout mice, the SCC group included 5 females and 5 males from a total of 4 litters; the FCC group included 7 females and 2 males from a total of 4 litters. Of the GSTM1 wild-type subjects in Experiment 2, 12 mice received social contextual conditioning (WT-Social, n=12) and 9 mice received food contextual conditioning (WT-Food, n=9). Wild-type mice receiving SCC included 5 females and 7 males from 3 litters, while wild-type mice receiving FCC included 3 females and 8 males from 2 litters.

Experiment 3: Effects of postnatal valproic acid treatment on social contextual conditioning

Experiment 3 (N=20) examined the effects of P14 valproic acid (n=11) or saline (n=9) on social contextual conditioning. Valproic acid subjects included 7 females and 4 males from a total of 8 litters, and saline subjects included 3 females and 6 males from a total of 3 litters.

III. RESULTS

Results of pilot study: Effects of home cage housing on primary measures of time in non-preferred bedding and crossovers

A pilot study prior to Experiment 1 examined the effects of strain (BALB/c or C57BL/6) and observation period (pretest or post-test) on temporal distribution of responses in novel bedding materials by adolescent mice housed between P22 and P29 in home cage isolate housing in familiar beta chip bedding. Results of this pilot study are depicted in Figures 1, 2 and 3. Figure 1 depicts time spent in nonpreferred bedding for BALB/c and C57BL/6 home cage control groups. A time of 300 seconds in non-preferred bedding would indicate no bedding preference. A two way repeated measures ANOVA on the effects of strain and observation period (pretest or post-test) revealed that neither strain ($F(1, 13) = 1.46$, $p > 0.05$) nor observation period ($F(1,13) = 2.89$, $p = 0.1128$) had an effect on the time spent in nonpreferred bedding. There was no interaction between strain and observation on time spent in nonpreferred bedding ($F(1, 13) = 2.89$, $p > 0.05$).

Figure 2 shows crossovers between beddings for BALB/c and C57BL/6 control groups. Although there was a significant main effect of strain on the number of crossovers between beddings ($F(1, 13) = 8.70$, $p < 0.02$), there was no effect of observation period on crossovers ($F(1, 13) = 1.86$, $p = 0.19$) or an effect of interaction between strain and observation period ($F(1, 29) = 0.01$, $p = 0.91$) on crossovers between beddings. Student-Newman-Keuls (SNK) post-hoc multiple pairwise comparisons revealed differences between strains; it was noted that for BALB/c mice, crossovers were no different during pretest and post-test ($p < 0.05$); likewise, for C57BL/6 mice, crossovers were no different during pretest and post-test ($p <0.05$); and, finally, BALB/c

and C57BL/6 groups did not differ from each other at either the pretest observation period ($p < 0.05$) or the post-test observation period ($p < 0.05$). Figure 3 shows horizontal movements during tests for BALB/c and C57BL/6 control groups. A two way repeated measures ANOVA on the effects of strain and observation period on horizontal movements revealed no main effect of strain ($F(1, 12) = 4.486$, $p > 0.05$), but a main effect of observation period on horizontal movement ($F(1, 12) = 7.11$, $p = 0.02$). No significant interaction occurred between strain and observation period ($F(1, 28) = 0.181$, $p > 0.05$). Post-hoc comparisons (SNK) revealed that horizontal movements were not different between BALB/c and C57BL/6 home cage control groups during the pretest ($p < 0.05$) or post-test ($p < 0.05$), but that within the C57BL/6 home cage control group, the number of horizontal movements was statistically different between pretest and post-test ($p < 0.05$). This difference was not observed in home cage control animals from the BALB/c strain ($p < 0.05$).

Experiment 1a

Observation period, but not strain, affects time in paired (non-preferred) bedding in social contextual conditioning groups

Experiment 1a assessed the effects of strain and observation period on the number and cumulative duration of approaches to paired bedding. Figure 4 shows time in paired bedding for BALB/c and C57BL/6 groups given social contextual conditioning. A two way repeated measures ANOVA revealed that there was no effect of strain on time in paired bedding ($F(1, 13) = 0.231$, $p = 0.63$), but a statistically significant effect of observation period on time spent in paired bedding ($F(1, 13) = 30.82$, $p < 0.0001$). There

was no statistically significant effect of interaction between strain and observation period on time in paired bedding ($F(1, 29) = 4.561$, $p > 0.05$). Results of SNK post-hoc comparisons revealed that for both C57BL/6 and BALB/c social contextual conditioning groups, time in paired bedding was significantly different between pretest and post-test (p's < 0.05), but that there was no difference during pretest ($p < 0.05$) or post-test ($p < 0.05$) between strains.

Figure 4b shows the effect of strain and conditioning on time in non-preferred bedding. A two way ANOVA using strain and conditioning group as between-subjects factors revealed a main effect of conditioning group ($F(1, 26) = 14.35$, $p < 0.001$) and a significant effect of strain ($F(1, 26) = 4.26$, $p < 0.05$), but no significant interaction between strain and conditioning group ($F(1, 29) = 3.28$, $p > 0.05$). Subsequent post-hoc analyses (SNK) were performed to further explore differences between groups and revealed that subjects in the home cage control condition differed from subjects in the social contextual conditioning group ($p < 0.05$), and that BALB/c subjects in the social conditioning group differed from C57BL/6 subjects in the social conditioning group ($p < 0.05$).

Figure 5 shows the number of crossovers between beddings for BALB/c and C57BL/6 groups given social contextual conditioning. A two way repeated measures ANOVA revealed no main effect of strain on crossovers between beddings ($F(1, 13) = 4.38$, $p > 0.05$), but a statistically significant effect of observation period on crossovers between beddings ($F(1, 13) = 27.5$. $p < 0.001$). No effect of an interaction between strain and observation period was observed for crossovers ($F(1, 29) = 2.52$, $p = 1.00$). Post-hoc comparisons (SNK) revealed that crossovers were not different between BALB/c and

C57BL/6 social contextual conditioning groups during the pretest ($p < 0.05$) or post-test ($p < 0.05$) observation period, and within BALB/c ($p < 0.05$) and within C57BL/6 ($p < 0.05$), crossovers differed between pretest and post-test observation periods.

Experiment 1b

Type of appetitive conditioning does not affect time in paired bedding

Figure 6 shows the effects of social (left panel) or food (right panel) contextual conditioning on time in paired bedding for BALB/c subjects. A two way repeated measures ANOVA was utilized to compare the effects of conditioning type and observation period on paired bedding time. There was no significant main effect of conditioning type on time in paired bedding ($F(1, 16) = 1.12$, $p > 0.05$). However, a statistically significant effect of observation period ($F(1, 16) = 49.29$), $p < 0.0001$) and a significant interaction between conditioning type and observation period ($F(1,35) = 5.45$, $p = 0.03$) were observed. Subsequent post-hoc pairwise multiple comparisons (SNK) showed that within the BALB/c social contextual conditioning group, paired time was different between test and pretest ($p < 0.05$); similarly, within the BALB/c food contextual conditioning group, paired time was different between test and pretest ($p < 0.05$). However, groups given FCC and SCC did not differ from each other during the post-test ($p < 0.05$).

Figure 7 shows the effects of social (left panel) or food (right panel) contextual conditioning on crossovers between beddings for BALB/c subjects. A two way repeated measures ANOVA compared the effects of conditioning type and observation period on crossovers. No significant main effect of conditioning type occurred ($F(1, 16) = 0.06$, $p >$

0.05), but a significant main effect of observation period on crossovers was observed ($F(1, 16) = 29.69$, $p < 0.0001$). No interaction occurred between conditioning type and observation period ($F(1, 35) = 0.428$, $p > 0.05$). Post-hoc comparisons found no differences between social and food conditioning at pretest ($p < 0.05$) or post-test ($p < 0.05$), but revealed differences between pretest and post-test for both social contextual conditioning (SCC), $p < 0.05$, and FCC ($p < 0.05$).

Strain does not affect time in paired bedding after food contextual conditioning

Figure 8 shows, for both BALB/c and C57BL/6, the effects of food conditioning on time in paired bedding. A two way repeated measures ANOVA was employed to evaluate the contribution of strain and observation period to time in paired bedding. No main effect of strain on paired time was observed ($F(1, 18) = 0.829$, $p > 0.05$), but observation period had a statistically significant effect on time in paired bedding ($F(1, 18) = 40.8$, $p < 0.0001$). No interaction between strain and observation period was observed for paired time ($F(1, 39) = 0.568$, $p > 0.05$). Post-hoc comparisons (SNK) revealed that BALB/c and C57BL/6 food contextual conditioning groups did not differ from each other during the pretest ($p < 0.05$) or during the post-test ($p < 0.05$), but within each strain, pretest and post-test scores on paired bedding time were different (p's < 0.05).

Strain and conditioning effects on crossovers in food contextual conditioning groups

Figure 9 shows, for both BALB/c and C57BL/6 groups, the effects of food contextual conditioning on crossovers between beddings. A two way repeated measures ANOVA was used to assess the effects of strain and observation period on crossovers. In

this analysis, both the main effect of strain ($F(1, 18) = 5.40$, $p = 0.03$) and the main effect of observation period ($F(1, 18) = 35.12$, $p < 0.0001$) were statistically significant. However, there was no observed interaction between strain and observation period ($F(1, 39) = 1.41$, $p = 0.25$). Results of subsequent post-hoc pairwise multiple comparisons (SNK) revealed that within group BALB/c and also within group C57BL/6, pretest and post-test crossovers differed significantly ($p < 0.05$), and that BALB/c and C57BL/6 groups did not differ from each other during the pretest ($p < 0.05$). However, during the post-test, BALB/c and C57BL/6 groups were different from each other ($p < 0.05$).

Experiment 2a

No effect of GSTM1 genotype on social contextual conditioning

Experiment 2a assessed the effects of genotype, for either GSTM1 knockout or wildtype animals, on social contextual conditioning. Figure 10 shows GSTM1 knockout and wildtype subjects given SCC. A two way repeated measures ANOVA used to analyze the effects of genotype and observation period on the time in bedding paired with social contact, revealed no main effect of genotype ($F(1, 20) = 0.012$, $p > 0.05$). However, the analysis showed a significant main effect of observation period on paired time ($F(1, 20) = 64.26$, $p < 0.0001$). No interaction was present between genotype and observation period ($F(1, 43) = 2.04$, $p > 0.05$). Post-hoc comparisons confirmed that within the GSTM1 knockout genotype, pretest and post-test paired time was significantly different ($p < 0.05$) and that this was true for the GSTM1 wildtype genotype as well ($p < 0.05$). However, there was no difference between genotypes at pretest ($p < 0.05$) or at post-test ($p < 0.05$).

Figure 10b depicts the effects of genotype and conditioning on responding above chance level (spending more than half of test session in non-preferred bedding) for GSTM1 knockout and wild-type animals. A two way repeated measures ANOVA revealed no significant effect of genotype ($F(1, 20) = 0.296$, $p > 0.05$) on chance level responding, but found a significant main effect of observation period ($F(1, 20) = 35.814$, $p < 0.0001$). No interaction was observed between genotype and observation period ($F(1, 43) = 0.296$, $p > 0.05$). Post-hoc comparisons revealed that for mice with GSTM1 knockout genotype, pretest and post-test scores were different. Likewise, for mice with GSTM1 wild-type genotype, pretest and post-test scores were different.

Figure 11 shows crossovers after social contextual conditioning for GSTM1 genotypes. A two way repeated measures ANOVA compared the effects of genotype and observation period on crossovers in subjects given social contextual conditioning. Results indicated that there was no main effect of genotype ($F(1, 20) = 3.87$, $p > 0.05$) on crossovers, but that there was a statistically significant effect of observation period ($F(1, 20) = 55.36$, $p < 0.00001$). No interaction was observed between genotype and observation period ($F(1, 43) = 0.017$, $p > 0.05$).

Experiment 2b

No effect of GSTM1 genotype on food contextual conditioning

Experiment 2b assessed the effects of genotype, for either GSTM1 knockout or wildtype animals, on food contextual conditioning. Figure 12 shows GSTM1 knockout and wildtype animals given FCC. A two way repeated measures ANOVA used to analyze the effects of genotype and observation period on the time in bedding paired with

palatable food, revealed no main effect of genotype ($F(1, 16) = 0.098$, $p > 0.05$), on time in paired bedding. However, the analysis showed a significant main effect of observation period on time in paired bedding ($F(1, 16 = 23.28$, $p < 0.0002$). No interaction was observed between genotype and observation period ($F(1, 35) = .0001$, $p > 0.05$). Results of SNK post-hoc pairwise multiple comparisons confirmed that within the wildtype food conditioned group, time in paired bedding was significantly different between pretest and post-test ($p < 0.05$). Likewise, within the knockout food conditioned group, time in paired bedding was significantly different between pretest and post-test ($p < 0.05$). However, there was no difference between wildtype and knockout groups during the pretest ($p < 0.05$) or during the post-test ($p < 0.05$).

Figure 13 shows crossovers as a function of food contextual conditioning for GSTM1 genotypes. A two way repeated measures ANOVA revealed no main effect of genotype on crossover behavior ($F(1, 16\_ = 0.749$, $p > 0.05$) and a statistically significant effect of observation period ($F(1, 16) = 45.78$, $p < 0.0001$). No interaction between genotype and observation period was observed ($F(1, 35) = 4.033$, $p > 0.05$). Post-hoc comparisons (SNK) showed that crossovers did not differ between genotype during pretest ($p < 0.05$) or during post-test ($p < 0.05$), within the food conditioned knockout group, pretest was different from post-test ($p < 0.05$), and within the food conditioned wildtype group, pretest was different from post-test ($p < 0.05$).

Experiment 3

Valproic acid impairs social contextual conditioning in BALB/c mice

Experiment 3 compared the effects of postnatal (P14) treatment with valproate (400 mg/kg) or saline on social contextual conditioning. Figure 14 shows the effect of toxicant on time in social contact-paired bedding for BALB/c mice. A two way repeated measures ANOVA was used to assess the contributions of toxicant (VPA or saline vehicle) and observation period (pretest or post-test) on time spent in paired, previously nonpreferred bedding. Results of the analysis show no primary effect of toxicant on time in nonpreferred bedding ($F(1, 18) = 0.791$, $p > 0.05$), but a statistically significant main effect of observation period on paired bedding time ($F(1, 18) = 44.903$, $p < 0.00001$). Importantly, a statistically significant interaction was observed between toxicant and observation period ($F(1, 39) = 8.81$, $p = 0.008$). Pairwise multiple comparisons (SNK) were employed to further assess the differences between groups at different observation periods, and revealed that saline and VPA groups were no different from each other in terms of time in paired bedding during the pretest ($p < 0.05$). However, the social contextual conditioning groups given saline or VPA were different from each other during the post-test ($p < 0.05$). Within the SCC saline group, time spent in paired bedding was different between pretest and post-test ($p < 0.05$), similar to the SCC_VPA group, in which paired time differed between pretest and post-test ($p < 0.05$).

Figure 14b shows responding above chance level (spending more than half of the testing session in the previously non-preferred bedding) that occurred for saline-treated, but not toxicant-treated, BALB/c mice given social contextual conditioning. A two way repeated measures ANOVA was used to assess the contributions of toxicant (VPA or saline vehicle) and observation period to responding above chance level. The analysis revealed significant main effects of both toxicant ($F (1, 18) = 7.16$, $p < 0.02$) and

observation period ($F(1, 18) = 40.70$, $p < 0.00001$), as well as a statistically significant interaction between toxicant and observation period ($F(1, 39) = 7.16$, $p < 0.02$).

Perseveration is increased after valproic acid treatment

The effect of valproic acid or saline on the tendency to perseverate, or respond solely to one feature of the test situation (such as allocating the majority of contact during every session to pine bedding, or allocating the majority of contact during every session to the left position of the chamber) was examined using a two way analysis of variance with strain (BALB/c or C57BL/6) and toxicant (VPA or saline) as between between subjects factors. (This analysis was performed on data from any BALB/c or C57BL/6 subject that had been given both toxicant or saline treatment and conditioning, and included 4 additional C57BL/6 subjects given postnatal treatment in a pilot study, whose conditioning scores have not been reported here.) Results of the analysis found no main effect of strain ($F(1, 58) = 0.27$, $p > 0.05$), but indicated there was a statistically significant effect of toxicant ($F(1, 58) = 16.82$, $p = 0.0001$) on perseveration. No significant interaction was observed between strain and toxicant ($F(1, 61) = 0.046$, $p > 0.05$). Post-hoc comparisons (SNK) revealed that C57BL/6 and BALB/c saline subjects did not differ from one another ($p < 0.05$), and that within C57BL/6, saline and VPA-treated subjects did not differ ($p < 0.05$). However, within BALB/c subjects, VPA and saline had statistically significantly different effects on perseveration ($p < 0.05$).

IV. DISCUSSION

Overview of results

Three studies were crafted to characterize the social contextual conditioning phenotype of adolescent mice: Experiment 1 compared the effects of strain in social contextual conditioning in BALB/c and C57BL/6 mice, Experiment 2 examined contributions of GSTM1 knockout or wild-type genotype to social contextual conditioning, and Experiment 3 examined the effects of postnatal toxicant treatment on social contextual conditioning in BALB/c mice. Striking in contrast to earlier reports of high approach of C57BL/6 mice (and low approach of BALB/C mice) to bedding paired with social contact (Panksepp and Lahvis, 2007, Panksepp et al., 2007) were our observations of strong social contextual conditioning in BALB/C as opposed to C57BL/6 mice. However, those studies differed from the present experiments in important ways, beginning with their usual operational definition of the social paired environment as four mice, two per gender, from the same litter. Instead, with the goal of defining the conditions minimally necessary to socially condition approach behavior in adolescent mice, we made repeated assessments of an individual animal's approach to a previously nonpreferred bedding after pairing sessions in that bedding with a single, non-littermate, peer mouse novel to the target subject mouse before the conditioning task. These studies, then, illuminate conditions under which mice from a strain known for low sociability demonstrate social reward that is equal in magnitude to the effects of contextual conditioning using food reward.

Relative to strong social conditioning in BALB/c saline-injected control animals, it was observed that valproic acid induced decreases in social contextual conditioning in

BALB/C mice, corroborating mounting evidence of social alterations produced by teratogenic exposure to valproic acid during neurobiological development (Schneider and Przewłocki, 2005; Schneider et al., 2006; Wagner et al., 2006; Yochum et al., 2008). The interactions between age, toxicant exposure and genetics reported in these models contribute to current thought on the etiology of autism, which implicates toxicant-induced oxidative stress in combination with genetically predisposed difficulties managing reactive oxygen species (Deth, Muratore, Benzecry, Power-Charnitsky, and Waly, 2008; Kern and Jones, 2006; Yochum and Wagner, 2009). However, although genetic polymorphisms of glutathione-S-transferase have been linked to autism (Daniels, Warren, Odell, Maciulis, Burger, Warren, and Torres, 1995; Jamain, Betancur, Quach, Philippe, Fellous, Giros, Gillberg, Leboyer, and Bourgeron, 2002; Williams, Mars, Buyske, Stenroos, Wang, Factura-Santiago, Lambert, and Johnson, 2007) and despite recent documentation of valproic acid-induced apoptosis in hippocampus of GSTM1 knockout mice (Yochum et al., 2010), we did not observe deficits in the social contextual conditioning of GSTM1knockout animals in relation to GSTM1 wildtype controls. Collectively these results suggest utility of the social contextual conditioning paradigm in modeling the impairments in social rapport observed in humans with autism spectrum disorders, and that further research is required in elucidating the link between neuropathological basis of impairments observed in glutathione-S-transferase polymorphisms and social reward. The next sections briefly and more specifically elaborate on the results described above.

Results of Experiment 1 suggest that for both BALB/c and C57BL/6 mice, both social contact and palatable food are rewarding in the contextual conditioning paradigm

Experiment 1a was performed in order to establish the minimum conditions sufficient to condition an increase in visit number and time to bedding paired with social contact during social contextual conditioning. For each individual, pairing took place in the bedding identified as least-approached during baseline assessment (pretest). The observation of a statistically significant increase in both time spent in paired bedding and in the number of crossovers between beddings during the post-test indicates that for both BALB/c and C57BL/6 groups, social contextual conditioning was effective. It is clear that, at least under these conditions, regular contact with a novel non-littermate peer is rewarding enough to condition approach to social contact-paired bedding. The significant increases in crossover behavior observed in both the BALB/c and C57BL/6 groups indicates that this crossover activity reflects conditioning and is not simply a result of maturation, given that this increase was observed only in conditioned groups, not in home cage controls during the previous pilot experiments. It is unclear at present why the magnitude of BALB/c social contextual conditioning exceeds that observed in the C57BL/6 group, and possible contributing factors of paradigmatic variations and strain differences will be later discussed.

Experiment 1b was performed as a positive control experiment to which the effects of social contextual conditioning could be compared. In this control experiment, mice were alternately housed in a previously nonpreferred (least approached) bedding sprinkled with palatable sweet cereal, or in their previously preferred (most approached)

bedding alone with no cereal (recall that *ad libitum* access to food and water occurred continuously throughout all experiments). Results indicate that both time in paired bedding, and crossovers between beddings, increased significantly after food contextual conditioning, and that this conditioning was observed in both BALB/c and C57BL/6 groups. When considered as a complement to Experiment 1a, the food contextual conditioning experiment adds to the evidence that both C57BL/6 and BALB/c strains appear equally capable of acquiring conditioned approach to bedding paired with appetitive reward that is either social or food-related. These results paved the way for the subsequent evaluation of conditioning effects in animals bred on a C57BL/6 background (Experiment 2) and for the evaluation of possible conditioning decrements in animals given postnatal valproic acid treatment.

Results of Experiment 2 suggest that for both GSTM1 -/- and GSTM1 +/+, social contact and palatable food are both rewarding in the contextual conditioning paradigm

Experiment 2a was performed in order to ascertain whether GSTM1 genotypes differed with respect to the capacity to undergo social contextual conditioning using the parameters minimally effective for mice in their background strain, C57BL/6. As depicted in Figures 10-13, social contextual conditioning and food contextual conditioning were both effective in establishing conditioned approach to paired bedding, as well as in inducing conditioned increases in crossovers between beddings. These results were not entirely expected, as a social impairment (or even a broader contextual conditioning impairment) would have been compatible with previous findings that

genetic polymorphisms in glutathione-S-transferase have been associated with both autism and increased vulnerability to toxicant related neuropathology. However, the intact conditioning observed here will provide an important baseline against which toxicant-induced alterations in contextual conditioning in GSTM1 knockout, compared to wildtype animals, can be compared in the future. If there is a detrimental interaction between genotype and valproic acid, for example, the results presented here suggest that a possible selective deficit in social as opposed to food contextual conditioning could be expected.

Results of Experiment 3 reveal detrimental impact of valproic acid on social reward in BALB/c mice

Perhaps the most important finding in these experiments is that valproic acid treatment decreased, as expected, the magnitude of social contextual conditioning in adolescent BALB/c mice. Although the saline and valproic acid treated groups did not differ in their temporal distribution of approach toward nonpreferred (least approached) or preferred beddings during the pretest, any conditioned increase in paired bedding approach was much less dramatic for VPA treated animals. However, the interesting finding that VPA attenuated, but did not abolish, social contextual conditioning was not entirely predictable, given the neuropathological ability of postnatal VPA treatment to induce apoptosis in the hippocampus and cerebellum (Yochum et al., 2008). Pilot studies inspired by the present experiments are currently underway to examine potential food contextual conditioning deficits in mice given postnatal toxicant treatment.

Perseveration was observed primarily in VPA-treated subjects

In the present experiments, the test situation constitutes a stimulus complex in which the position placement of cues varies daily; for conditioned groups, the factor controlling responding should be the local placement of the contextual cue (bedding previously paired with an unconditioned stimulus). This configuration means that the S+ (reinforcement associated cue) varies daily with respect to the S- (non-reinforcement associated cue). Every session was scored (even though only scores from Day 1 of testing are used for conditioning analyses), allowing analysis of the distribution of time with respect to specific physical and spatial characteristics of the stimulus context. It is possible to relate these types of analyses in animal models to the stimulus control problems observed in human autism, including behavioral rigidity, perseveration (Hollander, King, Delaney, Smith, and Silverman, 2003), and 'stimulus overselectivity', in which a small number of features controls responding to a more complex stimulus (Lovaas, Koegel, and Schreibman, 1979; Reynolds, Newsom, and Lovaas, 1974; Kriete and Noelle, 2008). Animal literature supports the use of tasks that differentiate between individuals with phenotypes related to perseveration, including tasks in which animals fail to respond to changed contingencies in learning tasks (Moy et al., 2007).

Tentatively, perseveration can be operationally defined as responding during every baseline and test session by (a) behavior describable in terms of a position preference (allocating the majority of time during baseline AND all testing sessions to either the right or the left side), or (b) behavior describable in terms of an inflexible bedding preference (allocating the majority of time during baseline AND all three testing sessions to only one type of bedding). Scoring responses during three consecutive daily

test sessions allowed observation of tendency to respond to either a feature of a particular bedding or a particular position. In this way perseveration can be measured as a pattern of responding that might interfere with either the acquisition or expression of conditioning, and is dissociable from chance performance. In the absence of conditioning, we observed at least two types of responding that appeared indifferent to the relevant contextual stimulus; that is, we observed several patterns in animals that did not respond during the test session by spending the majority of time in the paired (previously nonpreferred) bedding. Indifference with respect to the bedding type (corncob (C) or pine chip (P)) could produce a performance in which the sequences CPCP, PCPC, or PCCP, CPPC, or CPPP, or PCCC occur. Likewise, indifference with respect to bedding position could produce the sequences LRLR, RLRL, RLLL, LRRR, LRRL or RLLR. However, producing the sequence LLLL, RRRR, CCCC or PPPP on baseline and all testing days is unlikely unless the subject responds preferentially to a single feature of the stimulus complex.

The observation that these patterns produced by conditioning subjects occurred almost exclusively in valproic acid treated individuals is consistent with the possibility that animal models of autism could model some of the learning or response abnormalities observed in human autism (Moy et al., 2007). Whether these patterns reflect learning decrements or a formal construct consistent with human defined ‘perseveration’ that might reflect ‘stimulus overselectivity’ (Koegel and Wilhelm, 1973, Lovaas and Schreibman, 1971) is not as important as first describing conditions under which they are most likely to occur. In fact, stimulus overselectivity can be considered itself a type of learning decrement. Both could be equally likely to result from valproic acid treatment.

Like anxiety, stimulus overselectivity may be best described not as ‘a behavior’ but as a construct used to describe patterns of behavior evoked by certain stimulus conditions and behavioral history. (For a discussion of stimulus overselectivity and its relationship to stimulus control in preschool children including those with autism, readers are directed to Bickel, Stella and Etzel, 1984.) The ongoing studies to dissociate the effects of valproic acid on social conditioning from those on food conditioning should shed light on whether the ‘overselectivity’ or perseveration observed in VPA-treated subjects was a selective social-related deficit or a more general learning or memory related impairment.

Comparison of our paradigm with the social conditioned place preference paradigm described previously

In order to make a theoretical comparison between these results and those of Panksepp and Lahvis (2007), in which several experiments explored genetic variation contributing to social reward in the social conditioned place preference paradigm, several differences must first be pointed out. Our primary concern was adapting the social conditioned place preference paradigm for BALB/C mice in the context of our postnatal valproic acid model of autism. To assess the rewarding nature of social contact, we defined “social environment” as the presence of two mice of the same gender and strain background but from different litters, in contrast to Panksepp and Lahvis' inclusion of four littermates representing two genders. Another difference between their paradigm and ours is that while Panksepp and Lahvis (2007) included the presence of novel objects (PVC couplers) in both the social-paired and isolate-paired environments, as well as in the testing environment, the subjects in the present experiments were housed in bedding

material alone; no additional environmental cues were provided. Additionally, the present study's assessment of conditioned approach to bedding took place in the absence of a response cost to travel between the beddings, while the other authors used a three compartment apparatus in which travel to another bedding necessarily involves crossing a middle environment and crawling through an opening. A final important difference between the paradigms involves baseline assessment, as Panksepp and Lahvis (2007) assessed baseline preferences for the novel aspen or paper beddings in a group of animals as a single experimental manipulation. Because the resulting preferences did not appear systematically skewed to one or another bedding, Panksepp and Lahvis (2007) counterbalanced beddings paired with social contact or isolation across subjects. Instead, we assessed, for each individual, the initial approach and activity elicited by the two novel bedding materials, prior to conditioning.

In terms of results, the current study revealed the most robust contextual conditioning among BALB/c subjects, in a paradigm using novel same sex peers as social partners. This result was unexpected because Panksepp and Lahvis (2007) found BALB/c mice to be the least socially responsive of the four analyzed genetic strains in their study. Even so, their valid suggestion that strain differences impact the value of social reward is strengthened by the results of our studies. We reveal that under certain conditions, a 'socially unresponsive' strain of mice can be made to outperform other strains of mice in social contextual conditioning. Our observation of increased potency of this conditioning could be attributed in part to the increased salience of our unconditioned stimulus (social reward). Using only one mouse as a social partner in the absence of tangible objects could have facilitated attention to the peer mouse; further, increased attention to stimuli

increases the likelihood those stimuli will acquire behavioral functions. Theoretically, this explanation is compatible with a framework of viewing behavior in terms of alternative sets: to more fully describe behavior that is not fully explainable in terms of the contingencies into which it enters, a more complete description of alternative sets of relationships between behavior and environment (Goldiamond, 1974) may be required. That is, the absence of any additional cues (e.g., such as PVC couplers) in both paired and deprived environments could have enhanced the discriminability between the two conditions.

Although normally BALB/c mice have been characterized as less socially responsive than other strains (Panksepp and Lahvis, 2007, Panksepp et al., 2007, Moy et al., 2007; Brodkin, 2007), a very small number of studies have found unexpectedly more BALB/c sociability (discussed in Brodkin, 2007). The explanation for those anomalies may be found in genetic drift within laboratory environments, age of mice, or difficulties in discriminating between, or interpreting, affiliative versus aggressive approach behavior (2007). As mentioned previously, the present Experiment 1 yielded a higher degree of social contextual conditioning than expected in the BALB/C mice, and social contextual conditioning less robust than expected in the C57BL/6 mice, with respect to results of previous findings (notably Panksepp and Lahvis, 2007). As suggested above, several possibilities could explain these discrepancies. First, it is possible that there was a certain degree of social reward present but not measured in their study, or revealed by their paradigm. Supporting this possibility is the observation that between 30 and 50 percent of BALB/c mice in given groups were ‘nonresponsive’ and excluded from the study (2007). The current experiments’ absence of physical barriers to movement in the test chamber

may have afforded more opportunities to examine spatial allocation of social contextually conditioned approach behavior in BALB/c mice than in previous studies.

A related possibility is that for the BALB/C mice in Panksepp and Lahvis' (2007) and other social contextual conditioning studies using littermates as pairing partners, the presence of objects (PVC tubes) in the socially deprived condition deflated the aversive nature of isolation, that the presence of multiple littermates with whom much time had already been spent during development eroded the salience of the social condition, or both. Although BALB/C mice in our paradigm had subsequently been housed in social housing with littermates until weaning (PND21), their first encounter with novel beddings occurred in the presence of a novel peer mouse, under conditions which may have facilitated contextual conditioning.

Possible contributions of anxiety phenotype to social conditioning

A second kind of explanation for the high degree of social contextual conditioning expressed in the BALB/c SAL group in Experiment 1 has to do with the well-known anxiety phenotype of BALB/c mice (Moy et al., 2007; Brodkin, 2007; Kalueff, Ishikawa, and Griffith, 2008). Even if elicited by conditioning, an increase in movement is not necessarily indicative of appetitive reward or positive affect. However, arguing against an anxiety- or stress-related account of increased conditioning in BALB/c groups is the fact that normally, stress negatively impacts the ability of BALB/c, but not C57BL/6, mice to express contextual memories (Palumbo, Zubiletea, Cremaschia, and Genaroa, 2009). Furthermore, Brinks, deKloet, and Oitzl (2008) showed that corticosterone injections impair the ability of BALB/c mice to retain and subsequently express contextual

conditioning. It follows that stress or anxiety does not account for the performance of BALB/c on our task, because if conditioning was stress inducing rather than reward associated, we would likely have observed impaired, not enhanced, BALB/c performances.

Ultimately, although the social contextual conditioning paradigm purports to measure reward, further research is necessary to assess whether the neurobiological correlates of reward are, in fact, related to the conditioning sessions. Additional evidence against the argument that our paradigm led to artificially inflated movement in the BALB/C mice that was conditioned, but perhaps dissociable from social reward, could be obtained by ascertaining whether biomarker indices of stress or ultrasonic vocalizations associated with negative affect occur in the context of social conditioning (pairing) sessions. A neuropharmacological approach would also be useful in future studies, because it is highly likely that the effect on conditioned bouts of movement is pharmacologically dissociable from an effect on conditioned increases in duration of movement allocated to a space. Pharmacological manipulation of anxiety might affect the number of bouts of movement without affecting the distribution of behavior allocated to zones within a given spatial environment. That is, if the increases seen in both paired time and in number of crossovers were due primarily to a conditioned effect of social reward, administration of a benzodiazepene could attenuate movement without diminishing time spent in paired bedding.

A final point of concern is why Panksepp and Lahvis (2007) found such different (higher) rates of social contextual approach behavior in C57BL/6 group, while we found consistent but less dramatic social contextually conditioned approach C57BL/6 or

GSTM1 wildtype (on a C57BL/6 background) subjects. In the light of the explanatory power of the paradigmatic differences described above, it is feasible now to test the hypothesis that C57BL/6 may be more easily conditioned when multiple littermates, as opposed to a single novel peer, are social partners. Essentially, the questions addressed by previous social conditioned place preference studies might have been different enough from our experimental questions to suggest different results. Other studies asked whether mice would rather spend time in bedding paired with littermates or with isolation. Their conclusions have broadly indicated that mice from the C57 strain, to a greater degree than BALB/c, 'prefer' to spend time in the littermate-paired environment (Panksepp and Lahvis, 2007; Panksepp et al., 2007). The current studies, on the other hand, ask whether regular exposures to a non-littermate peer increase time in paired bedding. The resulting answer is a statistically significant affirmative for all strains tested, although visual inspection of the data may give the impression that C57BL/6 (including GSTM1 wildtype and GSTM1 knockout mice) spent less time in that bedding than predictable by previous studies alone.

The nature of VPA-induced alteration in social reward

Is a valproic acid-induced decrease in preference for social contact intractable, or is there a way to restore social interaction as rewarding? Since lower rates of social interaction are typically induced by valproic acid, one tenable explanation is that the toxicant alters the subjective value of engaging in social behavior. To the extent that social contextual conditioning was attenuated but not abolished by valproic acid, the social component of the task was rewarding enough to support conditioned approach to

social paired bedding. In fact, a slight deficit in both social and food contextual conditioning paradigms could be expected, in part because postnatal VPA induces apoptosis in the hippocampus (Yochum et al., 2008; Cheh et al., 2010). Presently, pilot studies are underway to address this hypothesis. Although more research is needed, pilot study results support the notion that in BALB/c mice, valproic acid may have a more detrimental effect on conditioned approach to social-paired bedding than on approach to food-paired bedding, even though both are tasks known to elicit hippocampal involvement. In order to more fully understand the extent to which VPA alters the subjective value of engaging in social behavior, pilot studies are comparing the effects of valproic acid on social interaction across a greater variety of housing conditions. It would be even more beneficial to measure neurophysiological correlates of reward to gain a more complete picture of how the affective experience of social interaction is changed by the toxicant.

Future studies could document other behavioral and neurobiological correlates of social reward experienced under valproic acid treatment by, for example, recording ultrasonic vocalizations. In this way, several recent studies have recorded neurobiological components of affective responses to rewards including sexual contact (Barfield, Auerback, Geyer, and McIntosh, 1979), rewarding brain stimulation (Burgdorf, Knutson, and Panksepp, 2000), social contact among adolescents (Panksepp et al., 2007) and self administration of cocaine (Barker et al., 2010). Barker et al. (2010) recently showed that the ultrasonic vocalizations produced by rats during self-administration of cocaine were dose dependent and corresponded to ranges associated with affective states. Rats on low dose self administered schedules emitted calls in the low, negative affect-associated,

range, while rats on high dose schedules emitted calls in the range associated with positive affect (Barker et al., 2010). This interesting finding supports using USV's as an empirical means to understand how the relationship between affective state and social reward seeking (e.g., Panksepp et al., 2007) may be affected by toxicant treatment.

As previously discussed, one mechanism by which by which valproic acid impairs embryonic cell differentiation is increasing the levels of intracellular reactive oxygen species (Na, Wartenberg, Nau, Hescheler, and Sauer, 2003). Significantly, Na et al. (2003) also showed restored cell differentiation if valproic acid was co-administered with vitamin E. Thus, in addition to elucidating the contributions of affective states to the neurobiology of social reward in the valpoic acid paradigm, future studies could evaluate the effects of co-administering free radical scavengers on toxicant-induced social conditioning deficits. Because oxidative stress is induced by social isolation and/or valproic acid, but social interaction is associated with increased protection against oxidative stress, it is suggested that social contextual conditioning could alter the response to oxidative stress. Future studies could address whether subjects that experienced the greatest degree of social contextual conditioning also experienced transient or long lasting reductions in oxidative stress, potentially measurable by markers for lipid peroxidation in urine. It would also be interesting to see whether Trolox (vitamin E derivative) treatment has a protective effect in combination with contextual conditioning, if co-administered with VPA. Such results would be consistent with data revealing protective effects of Trolox against neuropathological deficits induced by toxicant treatment (Cheh, Halladay, Reuhl, Polunas, Ming, and Wagner, 2007).

On the nonexclusivity of a relationship between 'social preference' and 'social reward'

Compatible with an effect of paradigmatic differences on the results of this study compared to others that used social conditioned place preference to assess social reward (Panksepp and Lahvis, 2007; Panksepp et al., 2007) is the notion that some greater degree of preference for social contact was present but not measured in previous studies for a variety of possible reasons discussed above. However, existence of preference must not necessarily be a prerequisite for social contextual conditioning. If one considers Panksepp et al.'s (2007) observation that social contact elicited affect-related USV's in BALB/c mice, and accepts the host of evidence that BALB/c seem to 'prefer' social contact to a lesser degree than other strains (Moy et al., 2007)—it is still clear that adolescent housing with a non-littermate peer may nonetheless have the capacity to establish and maintain socially conditioned approach behavior. In fact, this relates to theoretical argument that, like drugs, shock, and other stimulus events, social interaction is neither always rewarding, nor always aversive. Behavioral pharmacologists and behavior theorists have long theorized that aversive noxious stimuli can either maintain or suppress behavior depending on the schedule of stimulus delivery. For example, Kelleher and Morse (1969) suggested that electric shock could maintain positively reinforced performances. In monkeys given experience with shock-postponement schedules in recent behavioral history, fixed interval schedules of electric shock stimulus delivery can engender accelerated positively reinforced performances (McKearney 1968), despite the better-known tendency of organisms to avoid shock as a stimulus (Azrin, Hutchinson, and

Hake, 1966). It is well-established, then, that preference or liking for a stimulus may be a transient contextually dependent phenomenon.

In the human literature, self-reported preferences for events can be unrelated to the ability of those events to control or reinforce performances as Bekker-Pace (2006) observed in adult humans. In the study by Bekker-Pace (2006), after human participants self-identified 'preferred' and 'nonpreferred' sound clips, the quality of being identified as 'preferred' or 'nonpreferred' did not necessarily predict whether that stimulus would be successful in maintaining behavior. In theory, social behavior presents no exception to these arguments, because similar to a food or drug stimulus or a shock experience, it is impossible to arbitrarily designate social interaction as appetitive and social isolation as aversive. Whether a given encounter with social interaction or isolation is motivating or demotivating depends on variables such as the behavioral history of the organism (Dyer and Southwick, 1974), deprivation with respect to social interaction (Deak and Panksepp, 2006; Beatty, Dodge, Dodge, White, and Panksepp 1982), current drug context (Soffie and Bionchart, 1988; Deak and Panksepp, 2006), and factors in the nature of the social interaction experience itself (Varlinskaya, Spear and Spear, 1999). Because these factors contribute to whether the affective response to the social interaction is one of positive or negative valence, they also contribute to measurable neurobiological changes that occur during the expeirence. For example, regional brain opioid receptor binding is altered due to social manipulations (Vanderschuren et al., 1995). We structured the social contextual conditioning experience to be regular, predictable, and motivating: results indicate that, given the opportunity to divide time between two beddings that had been paired with either social interaction or with social isolation, the majority of BALB/c animals tested

spent more time in the bedding paired with social interaction (Experiment 1). This effect was blunted, but not necessarily abolished, by postnatal treatment with valproic acid (Experiment 3). It remains to be observed whether social interaction is as negatively affected by postnatal toxicant treatment (e.g., Yochum et al., 2008) after a behavioral conditioning history in which social contextual conditioning occurred.

In view of the interpretation that correspondence between preference and reinforcing ability of stimuli is not necessary for conditioning (Bekker-Pace, 2006), the results of the present studies have interesting implications for autism treatment literature. Preference is no small matter in developmental disabilities research, in which much effort has been devoted to establishing the best methods and practices for assessing preference (DeLeon and Iwata, 1996; DeLeon, Iwata, Goh and Worsdell, 1997; Cannella, O'Reilly and Lancioni, 2005; Romaniuk and Miltenberger, 2001). Sometimes referred to as preference assessment studies, these experiments examine variables such as the sequence of stimuli encountered (Conyers, Doole, Vause, Harapiak, Yu and Martin, 2002), duration of interacting with them (Carter, 2001), and whether they are presented to the organism in sequence or simultaneously (DeLeon and Iwata, 1996). In these studies as well as human research with adults without disorders (Bekker-Pace, 2006), preference among versus between stimuli, or the extent to which they are approached, is often affected by the availability and quantity and quality of available concurrent choices. It is suggested that this area be productively viewed as a potential research node, at which the autism treatment literature and the animal sociability literature could learn from each other. In the animal literature, commune nest studies (in which multiple litters are raised in the communal environment experienced by many rodents in the wild), are an example

of a kind of study evaluating effects of multiple and variable perinatal social experiences on the richness and complexity of an individual animal's life (Schradin and Pillay, 2004) and neural development (Branchi, 2009).

Another translatable finding in these results is that potential differences between C57BL/6 and BALB/c in the ability of siblings versus novel peers to facilitate social conditioning has potential implications for peer studies in children from same or different families. Although the current studies only examined pairs from matched toxicant conditions, pairings between children with autism and typically developing peers are more common (Roeyers, 1996; Goldstein and Cisar, 1992; Laushey and Heflin, 2000; also see Weiss and Harris, 2001) when increasing the density and richness of therapeutic environmental interactions is a target of treatment. It follows that future studies investigating the social interactions of treated mice after social contextual conditioning should assess the effects of different combinations of intact or toxicant-exposed subjects, and that these sorts of studies could provide prospective information on biochemical and neurodevelopmental analogs to these kinds of interactions in people.

Considerations in selecting the dependent variables

The dependent variables in these experiments, the duration (called nonpreferred time or paired bedding time) and number of approach bouts distributed between peer- and isolation-paired bedding materials (sometimes called crossovers or switching), were carefully chosen given an analysis of what measures are likely to be reliably and sensitively affected by the behavioral history provided by 8 days of social contextual conditioning. Conditioning history predicts behavior under extinction (Anderson, 2000;

Ferster and Skinner, 1957), to the extent that responding under a certain set of environmental relationships establishes mathematical patterns in the behavioral repertoire. (The amount of information in the behavior stream that becomes more predictable and related to known variables (e.g., Schoenfeld and Farmer, 1970; Morse and Kelleher, 1970), increases after conditioning and is quantifiable by, for example, the entropy equation (Shannon 1948; Shannon and Weaver, 1949). Some examples of predictable events we observed in the behavior streams of mice of every strain under social housing conditions are huddling, rearing, facial sniffing, social grooming, sleeping in piles, chasing, anogenital sniffing, close following and social proximity, all observed at some point during descriptive analysis of social behavior that occurred during the last social pairing session during conditioning.) Switching, or behavior that is described as occurring during the interresponse time, accounts for a substantial portion of choice behavior (Davison, 2004) and is a response susceptible to operant control (Baum, 1982; Todorov, 1971; Pliskoff, Cicerone, and Nelson, 1978). Thus, given the selection of paired bedding time and crossovers between beddings, testing under extinction conditions produced reliable increases in both of these measures for most conditioned groups of mice.

On the theoretical relationship between autism, contextual learning, and stimulus control

Animal models manipulating genes thought to be associated with autism have revealed deficits in contextual fear learning (Qiu, Korwek, Pratt-Davis, Peters, Bergman, and Weeber, 2006; DeLorey, Handforth, Anagnostaras, Homanics, Minassian,

Asatourian, Fanselow, Delgado-Escuela, Ellison, and Olsen, 1998; Belmonte, Allen, Beckel-Mitchener, Boulanger, Carper, and Webb, 2004). However, although hippocampal abnormalities have also been documented in persons with autism (Aylward, Minshew, Goldstein, Honeycut, Augustine, Yates, Barta, and Pearlson, 1999; Saitoh, Karns, and Courchesne, 2001), human evidence for an autism-related impairment in contextual learning is mixed. Theoretical evidence for hippocampal impairment in autism comes from Frith's theory of weak central coherence, which suggests that central coherence, or the loss of separate identity of features in favor of a coherent, meaningful bound representation, tends to be impaired in autism (Frith, 2001; Castelli, Frith, Happe, and Frith, 2002). In another line of research, the observation of stimulus overselectivity in children with autism (Reynolds et al., 1974; Kriete and Noelle, 2008) has been posited as behavioral evidence for a possible decrement in contextual learning (DeLong, 1992). As previously mentioned, stimulus overselectivity is defined as control of responding to a complex stimulus by a limited number of components, or responding to irrelevant features or dimensions of the stimulus (Lovaas, Schreibman, Koegel, and Rehm, 1971). However, Bickel, Stella and Etzel (1984) allowed that instead of labeling a learner as 'overselective' or labeling stimulus control as 'restricted', a comprehensive analysis of the hierarchy of stimuli controlling behavior could be used to suggest treatments to ameliorate targeted deficits.

Similar to the importance Goldiamond (1974) placed on considering the role of alternative sets of behavior in controlling responses is the notion that stimuli which are *present,* but not responded to, are nonetheless capable of exerting conditional control over responses (Bickel et al., 1984). This observation has been made with respect to

children with autism who also exhibited characteristic responding described as overselectivity (Cook and Rincover, 1978). In this vein, it is possible that by making the procedural modifications to the BALB/C social contextual conditioning paradigm, we increased the likelihood of attention to social contact as a relevant aspect of the social paired environment. Our attempts to make social contact more salient for BALB/c subjects by reducing the number of peer partners to one and eliminating all tangible stimuli except bedding from the conditioning chambers, is a tactic consistent with recommendations by Bickel, Stella and Etzel (1984) for situations in which an experimenter desires for a particular stimulus element low in the hierarchy is to gain control of behavior. Bickel et al. (1984) suggested that removing elements with a higher position in the hierarchy could result in the desired gaining of stimulus control by a lower element.

On the continued utility of animal modeling of social behavior

How ethologically valid are comparisons between rodent social behavior and that of people? Both young and older persons with autism spectrum disorders have specific types of impairments in social interaction. Social interaction needs to be discussed more specifically if those using and contributing to the animal model literature are to appreciate the critical nature of clarifying how, *for a given individual*, the impairment affects functioning and is maintained. Problems in understanding the meaning of an other's expressed affect is one of the most persistent features of social deficits. This feature is related to the oxytocin system, the neurochemical pathway for neurotransmitter and hormonal interactions that support pair bonding (Insel and Hulihan, 1995), feeding

and bonding with an infant, and even inter-species attachment between pets and their owners. That is, oxytocin system functioning, including oxytocin receptor density and distribution, is genetically related to pair bonding, degrees of liking and engaging in social interaction between oneself and others (Hammock and Young, 2006). Acting as a drug, oxytocin may facilitate social memory, facilitates face recognition (Marsh, Yu, Pine, and Blair, 2010), and trust between human individuals. Meanwhile, rodent studies suggest oxytocin reverses the social interaction deficts and brain abnormalities caused by early social isolation or drug exposure (Lee, Brady, Shapiro, Dorsa, and Koenig, 2007). In fact, at the base of the diverse specific social deficit present in autism may be mechanisms in common with those responsible for difficulties in attending to, perceiving the meaning of, or responding with approach toward affective communicative signals from peers in the environment-- the problems experienced by rodents deprived of opportunity to socially interact sufficiently with peers.

Animal studies can be useful to study human social behavior because an understanding of the basis of conserved mechanisms, like those shared by humans and diverse species that underlie similar aspects of social approach behavior, can be gained by comparing their shared features, even though the systems have evolved in the context of different species. For example, although the rodent oxytocin system subserves the highly olfactory dependent communication system (Ferguson, Aldag, Insel, and Young (2001), in humans, the socially relevant effects of oxytocin are not limited to those related to the olfactory system. In an interesting evaluation of intranasal oxytocin's effects on human behavior, Keri and Benedek (2009) found that it reduced the stimulus threshold for detecting biological, but not nonbiological, motion presented by a computer screen.

The authors interpreted this effect as evidence of the ability of oxytocin to enhance recognition of socially relevant stimuli. In fact, oxytocin is involved in processing the negative or positive emotional valence of faces (Gamer, Zuroski, and Buschell, 2010), and increases visual attention to eye gaze of others (Gamer et al., 2010; Guastella, Mitchell, and Mathews, 2008). Recently, Guastella, Einfeld, Gray, Rinehart, Tonge, Lambert, and Hickie (2010) found that intranasal oxytocin improved emotion recognition on the Reading the Mind in the Eyes Task, for 12-15 year old individuals with autism spectrum disorders.

Potential ability of social contextual conditioning to ameliorate deficits in social interaction induced by VPA

Dawson (2008) and others have discussed the possibility that intensive and early behavioral intervention on social and communication skills, which serves as the best researched empirically validated treatment for the behavioral deficits in autism to date, may produce observable effects on behavior due to neural plasticity mechanisms. One major site of neural plasticity, affecting the reorganization of the neural pathways subserving learning and memory, is the dentate gyrus region of the hippocampal formation, which is the locus of morphological changes following environmental enrichment. Furthermore, although toxicants such as VPA affect the hippocampus and alter performance on hippocampal dependent tasks (Wagner et al., 2006; Yochum et al., 2008), the gains experienced by children provided with early and intensive behavioral intervention (Fenske et al., 1985) suggest that the neurodevelopmental pathology of autism may not always be intractable. Similar results in rodent models of autism, in

which environmental enrichment ameliorated deficits in performance originally induced by VPA exposure (Schneider et al., 2006) may suggest that better understanding of biomarkers in early neurodevelopment could lead to treatment improvements by targeting neural plasticity-related and other mechanisms supporting empirically validated improvements in behavioral performance.

Almost seventy years after Kanner described 'autistic disorders of affective contact' (1943), psychologists continue to struggle to understand the links between one's own affect, and the ability to recognize and interpret an other's affective state (Moody, McIntosh, Mann and Weisser, 2007; Beall, Moody, McIntosh, Hepburn, and Reed, 2008; McIntosh, Reichmann-Decker, Winkielman, and Wilbarger, 2006). Although research is not necessarily approaching a “cure” for neurodevelopmental deficits, seeking to understand the basis of social differences forces science closer to their appreciation, which may someday be applied to neurodevelopmental disorder prevention and even remediation. The results of the present experiments contribute an instance of within-subject comparison of social reward in individuals.

Given the ability of environmental enrichment to reverse behavioral alterations produced by VPA in rats, the model may constitute an enrichment experience. This hypothesis is being tested by follow up studies exploring the interaction between behavioral history and valproic acid on subsequent encounters with novel partners in the social interaction test. The social facilitation observed after social conditioning in these pilot studies to date supports the continued examination of the interaction between neurodevelopmental toxicant exposure and early enriching experiences. Amelioration of behavioral deficits via environmental enrichment is a valid rodent analogue to the human

autism treatment literature, in which child "confederates", or helpers taught to persist in initiating to their peers with autism eventually produce responses that can be reinforced by the natural environment (e.g., Koegel and Koegel, 1995).

On the implications of these results for policy

Other researchers have pointed to prospective studies as useful to document the ontogeny of visual attention shifts that might be precursors to disturbances in joint attention observed in autism spectrum disorders from early childhood to adulthood (Klin, Jones, Schultz, Volkmar, and Cohen, 1995). Current research supports the utility of prospective studies that follow developmental trajectories of children at risk for autism spectrum disorders. For rodents, adolescence appears to be a critical and malleable time for changing and establishing preferences for social interaction potentially subserved by neural plasticity. For children, a similarly critical period for social interaction may be from birth to two or three, given the evidence that suggests earlier is better, in terms of when to provide intensive intervention on social skills. Much as Hart and Risley (1995) documented overwhelmingly that the number of words heard by children before a certain age were intractably related to much later outcomes on tests of reading and verbal comprehension ability, it is suggested that prospective studies could keep track of weekly or monthly opportunities to interact socially with others, for children at risk for ASDs. Early observations using prospective analyses of children with autism suggest that avoidance of eye contact and failure to engage in joint attention and imitative games are sometimes observable before the official diagnosis of autism (Dawson et al., 2000; Werner and Dawson, 2005). Data suggest that children given early intensive training on exactly these variables, eye contact etc, joint attention, etc, can sometimes make

substantial gains in treatment. Thus, more prospective studies are supported by data gleaned from the current experiments. Experimental analogues of these human studies should continue to study, in the context of interactions between age, environment and genetic susceptibility, how social interaction and sociability is related to the environmental, behavioral and genetic histories of individual animals. As others have noted, basic research is still necessary to understand the neurobiological basis of that characteristic fundamental to most behaving animals: rapport with others.

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FIGURE LEGENDS

Figure 1: Results of pilot study. For BALB/c and C57BL/6 groups, 8 days of home cage housing in standard bedding did not increase the amount of time spent in non-preferred (least approached) bedding.

Figure 2: Results of pilot study. For BALB/c and C57BL/6 groups, 8 days of home cage housing did not significantly increase the number of crossovers between beddings.

Figure 3: Results of pilot study. For C57BL/6 but not BALB/c mice, 8 days of home cage housing in standard bedding increased the number of horizontal movements during the post-test ($p < 0.05$).

Figure 4: In Experiment 1a, social contextual conditioning increased time in non-preferred bedding for both BALB/c and C57BL/6 strains.

Figure 4b: Effect of strain and conditioning were assessed on time in non-preferred bedding, using difference scores in which the number of seconds in non-preferred (paired) bedding during the pretest, is subtracted from the number of seconds spent in the paired bedding during the post-test. Home cage control subjects differed from socially conditioned subjects ($p < 0.05$), and within social conditioning groups, BALB/c mice were different from C57BL/6 mice ($p < 0.05$).

Figure 5: In Experiment 1a, social contextual conditioning increased crossovers between bedding for both BALB/c and C57BL/6 strains.

Figure 6: In Experiment 1b, both food and social contextual conditioning increased time in non-preferred bedding for BALB/c mice ($p < 0.05$).

Figure 7: In Experiment 1b, both food and social contextual conditioning increased crossovers between bedding for BALB/c mice ($p < 0.05$).

Figure 8: In Experiment 1b, food contextual conditioning increased time in non-preferred bedding for both BALB/c and C57BL/6 mice ($p < 0.05$).

Figure 9: In Experiment 1b, food contextual conditioning increased crossovers between beddings for both BALB/c and C57BL/6 mice ($p < 0.05$). There was a significant effect of strain on crossovers ($p < 0.05$).

Figure 10: In Experiment 2, social contextual conditioning increased time in non-preferred bedding for both GSTM1 -/- (knockout) and +/+ (wild-type) mice ($p < 0.05$).

Figure 10b: In Experiment 2, conditioning, but not genotype, resulted in responding that differed from chance levels in GSTM1 mice. Social contextual conditioning resulted in responding above chance level for both GSTM1 -/- (knockout) and +/+ (wild-type) mice.

Figure 11: In Experiment 2, social contextual conditioning crossovers between beddings for both GSTM1 -/- (knockout) and +/+ (wild-type) mice ($p < 0.05$).

Figure 12: In Experiment 2, food contextual conditioning increased time in non-preferred bedding for both GSTM1 -/- (knockout) and +/+ (wild-type) mice ($p < 0.05$).

Figure 13: In Experiment 2, food contextual conditioning increased crossovers for both GSTM1 -/- (knockout) and +/+ (wild-type) mice ($p < 0.05$).

Figure 14: In Experiment 3, postnatal treatment with valproic acid (VPA) attenuated social contextual conditioning in BALB/c mice ($p < 0.05$). SAL and VPA were different during the post-test ($p < 0.05$), but not during the pretest.

Figure 14b: In Experiment 3, postnatal toxicant treatment reduced the amount of responding that occurred chance levels in BALB/c mice given social contextual conditioning.

Figure 15: In Experiment 3, postnatal treatment with valproic acid (VPA) attenuated social contextual conditioning of crossovers in BALB/c mice ($p < 0.05$).

Figure 16: Postnatal treatment with valproic acid (VPA) induced perseveration, or responding primarily (during four consecutive test sessions) to a single aspect of the test complex (such as responding repeatedly to a ‘position preference’ or ‘bedding

preference'). Right panel shows six additional C57BL/6 animals injected with VPA as part of an otherwise-unreported pilot experiment.

Figure 1: Home cage controls (time in bedding)

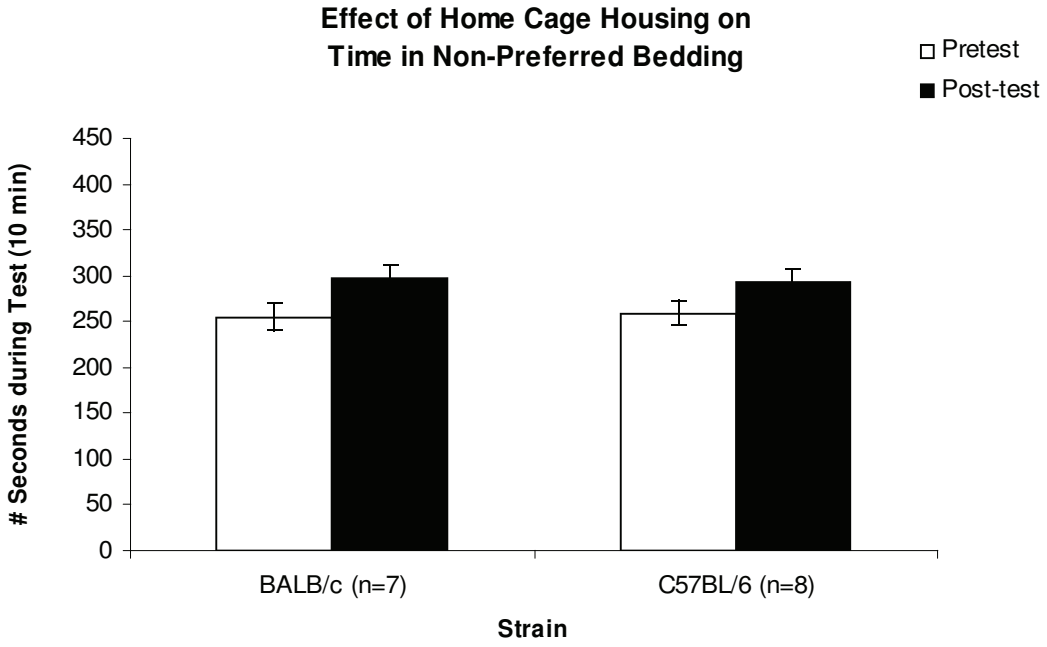


Figure 2: Home cage controls (crossovers between beddings)

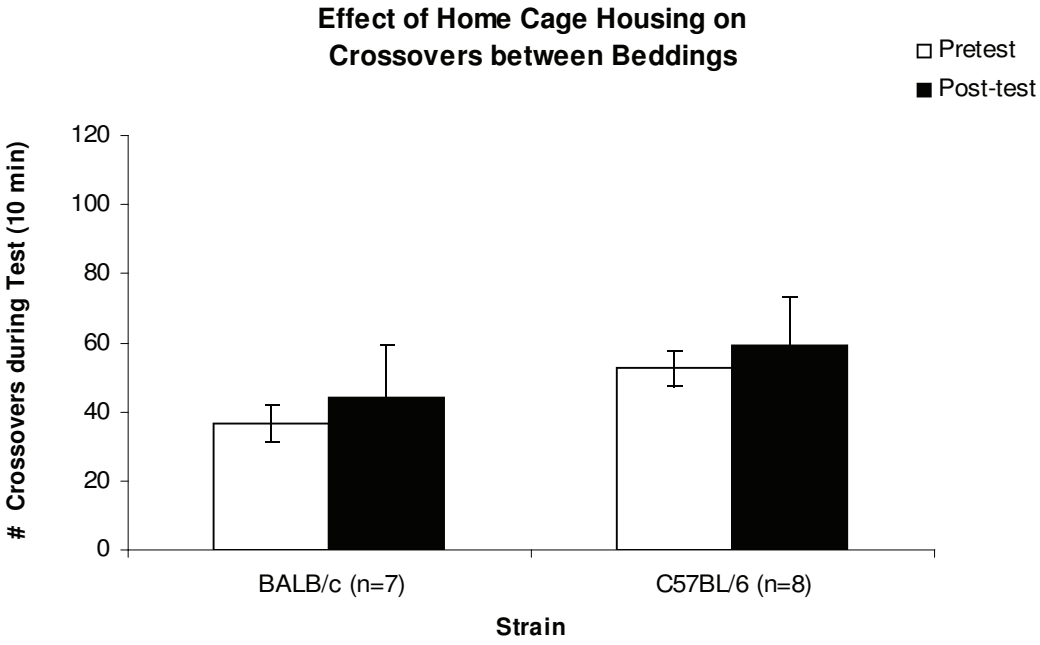


Figure 3: Home cage controls (horizontal movements)

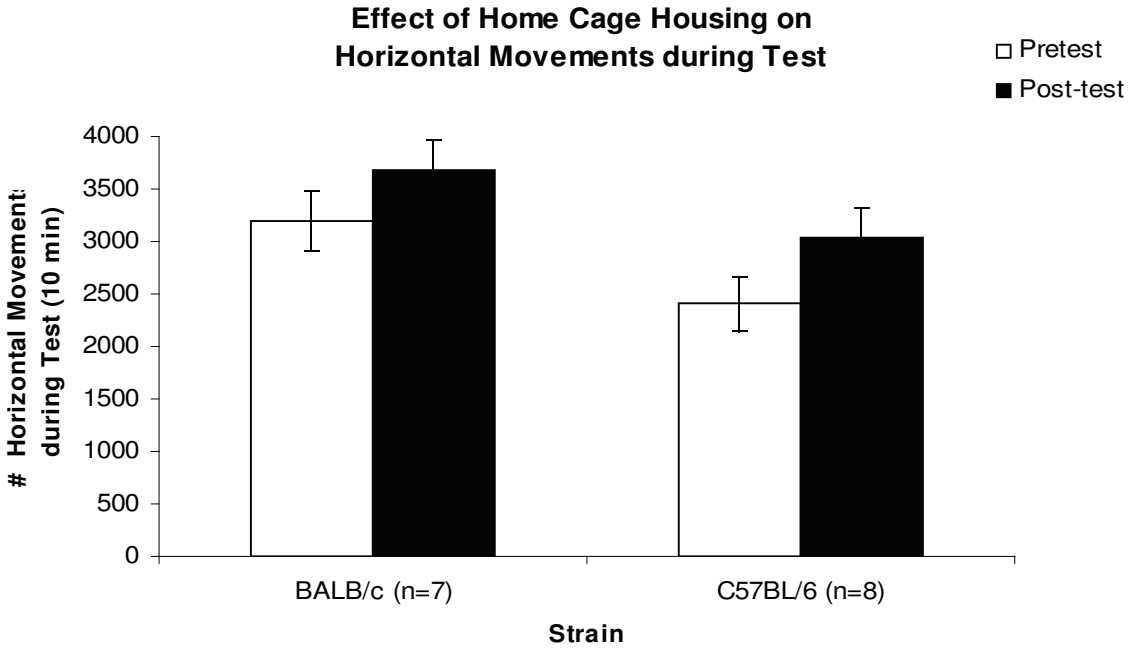


Figure 4: Social conditioning in BALB/c and C57 (time in bedding)

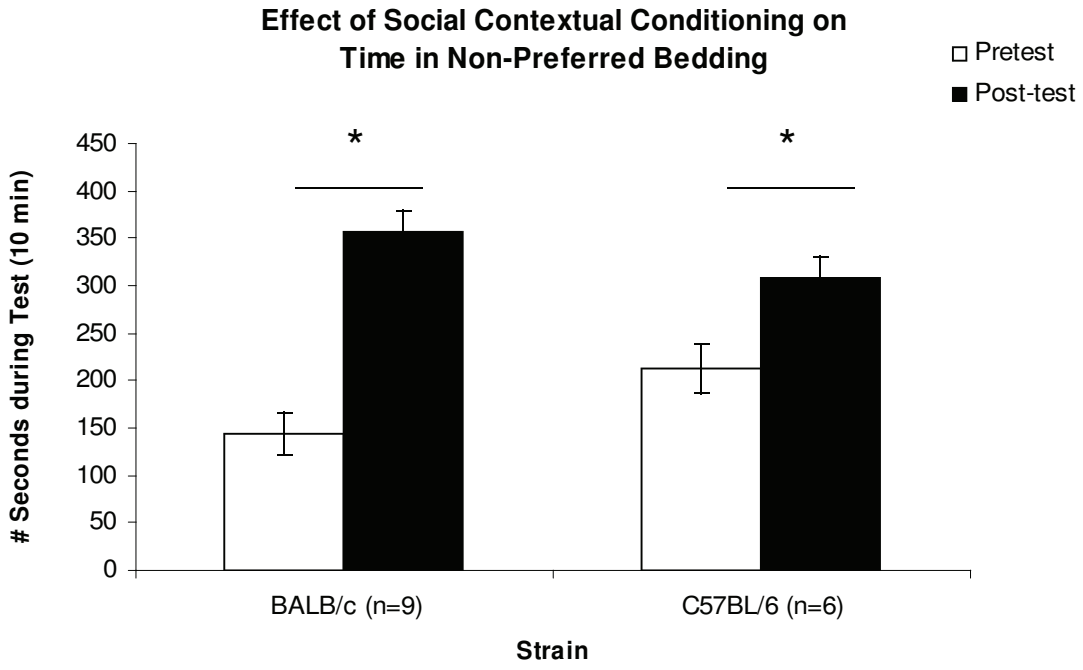


Figure 4b: Social conditioning in BALB/c and C57 (difference scores)

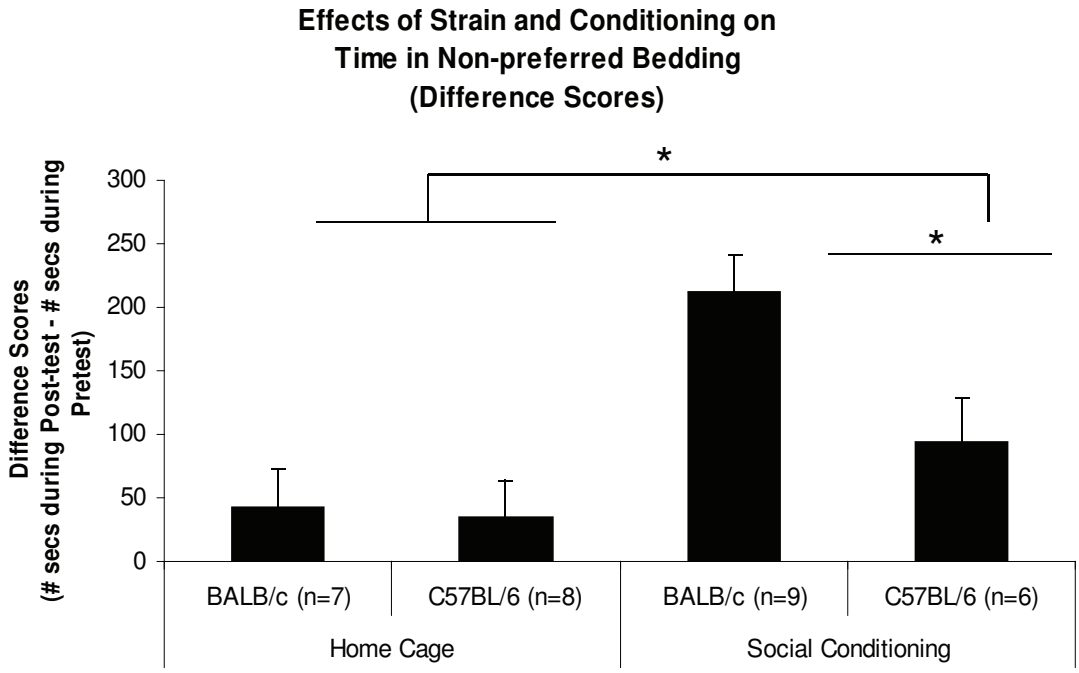


Figure 5: Social conditioning in BALB/c and C57 (crossovers between beddings)

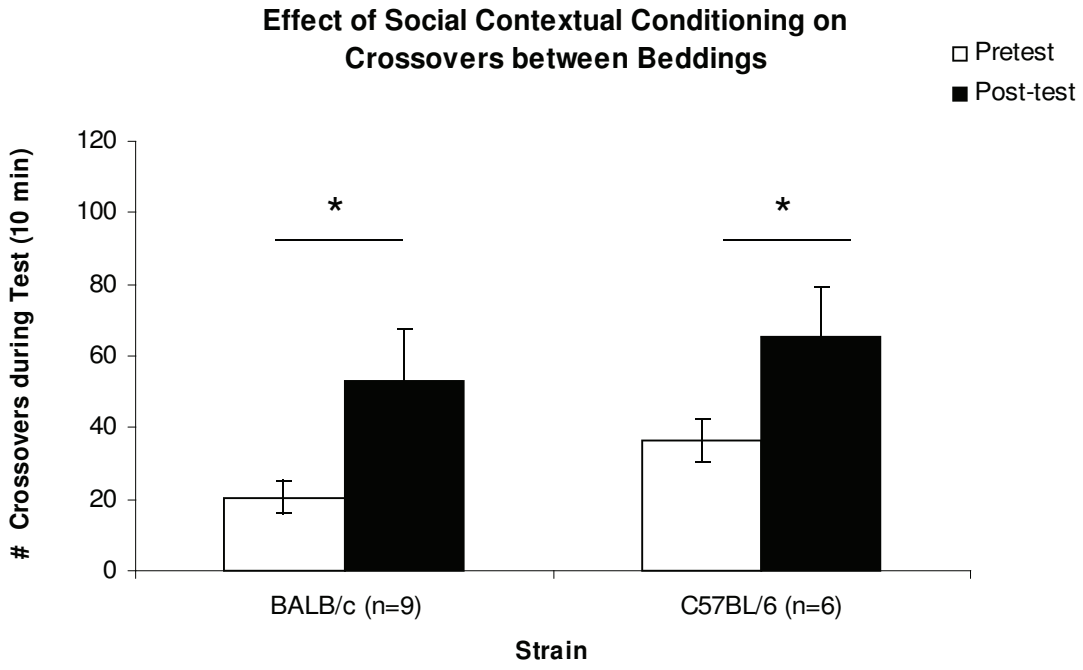


Figure 6: Food and social conditioning in BALB/c (time in bedding)

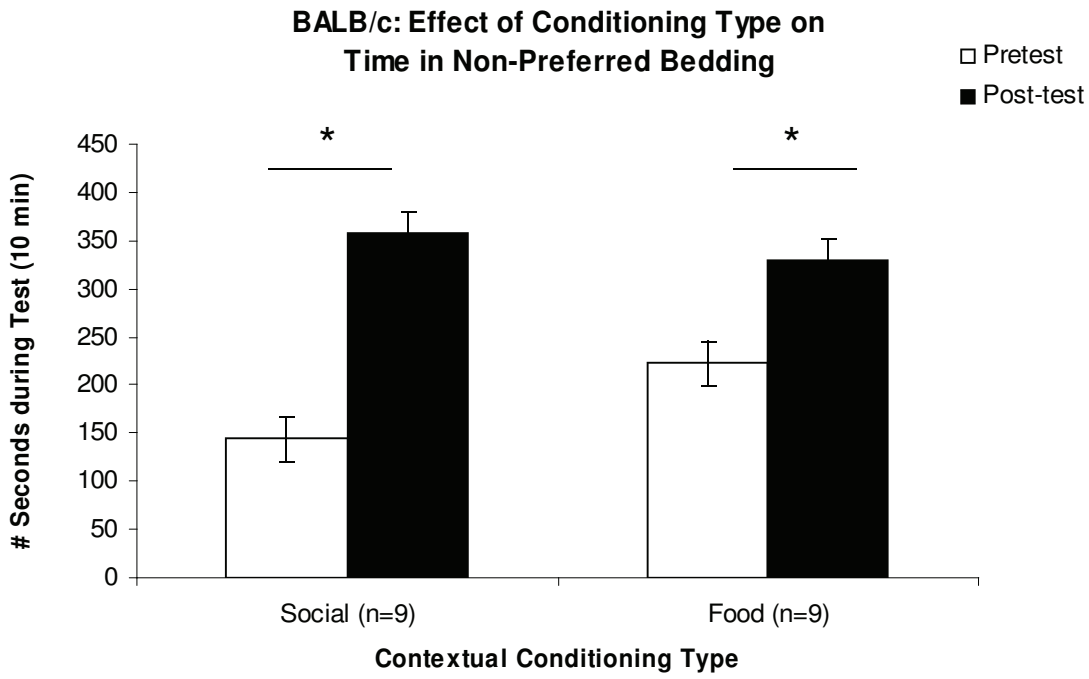


Figure 7: Food and social conditioning in BALB/c (crossovers between beddings)

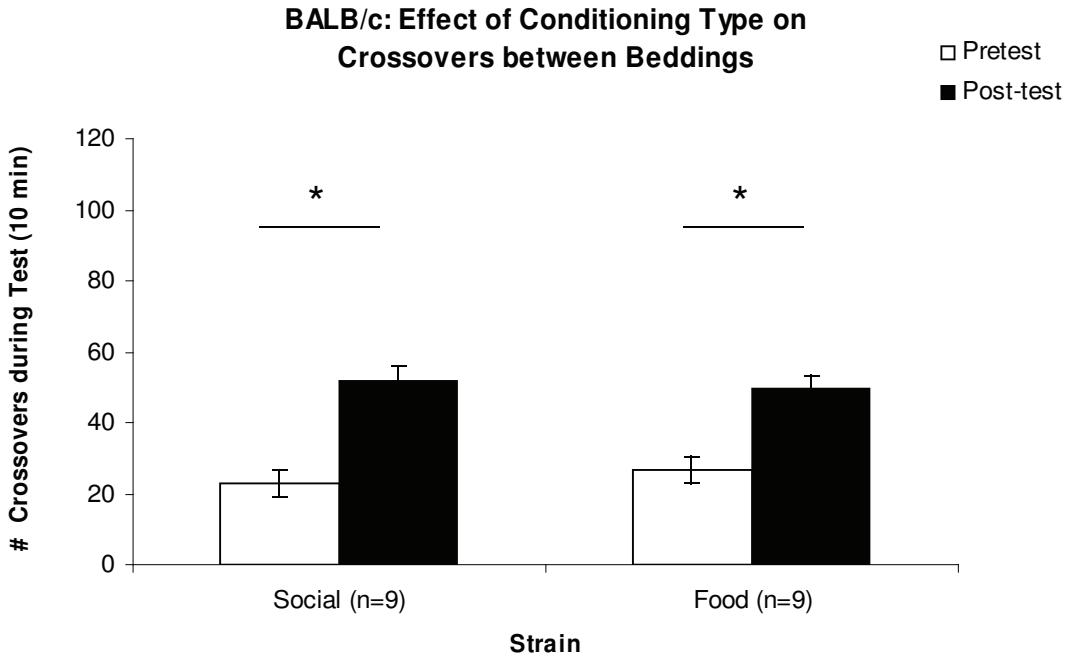


Figure 8: Food conditioning in BALB/c and C57 (time in bedding)

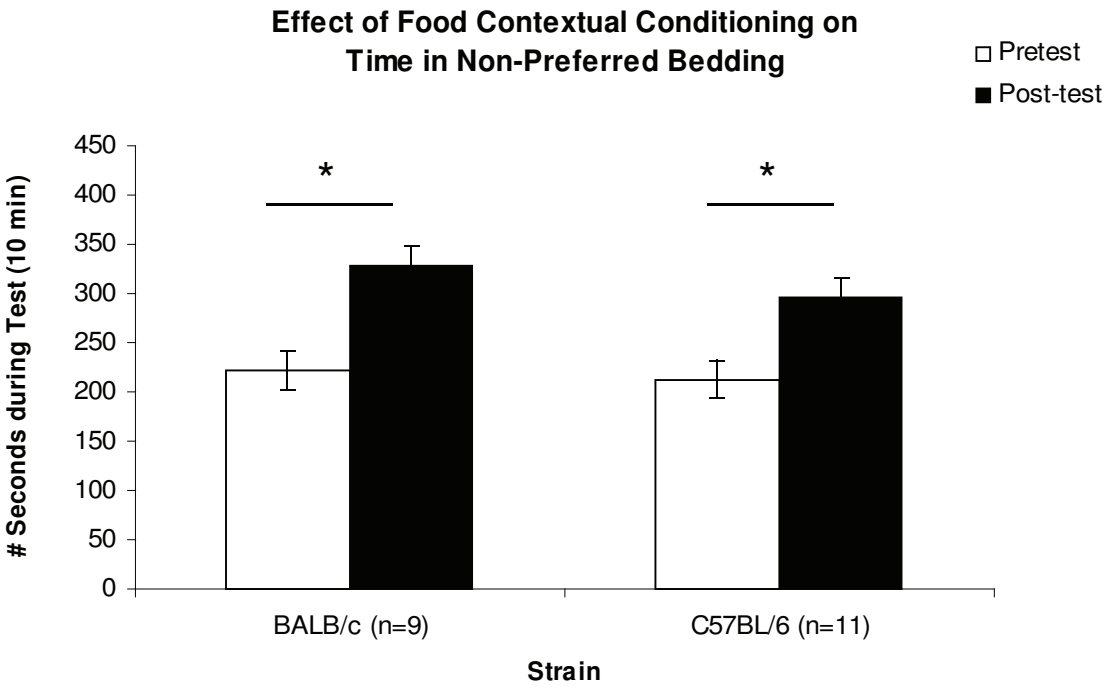


Figure 9: Food conditioning in BALB/c and C57 (crossovers between beddings)

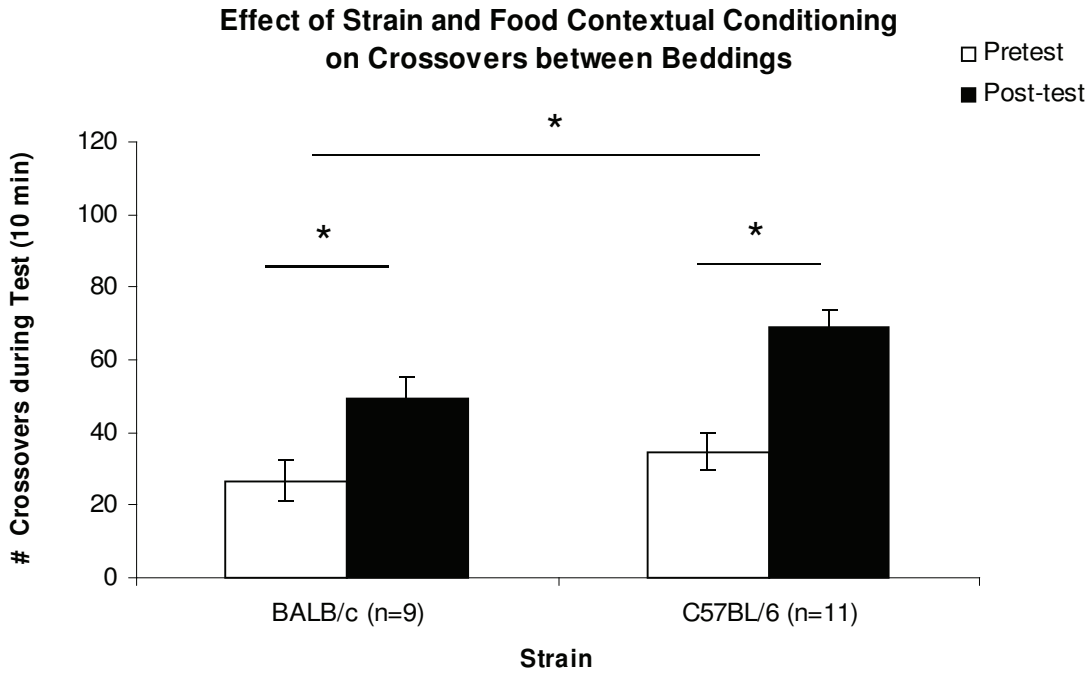


Figure 10: Social conditioning in GSTM1 (time in bedding)

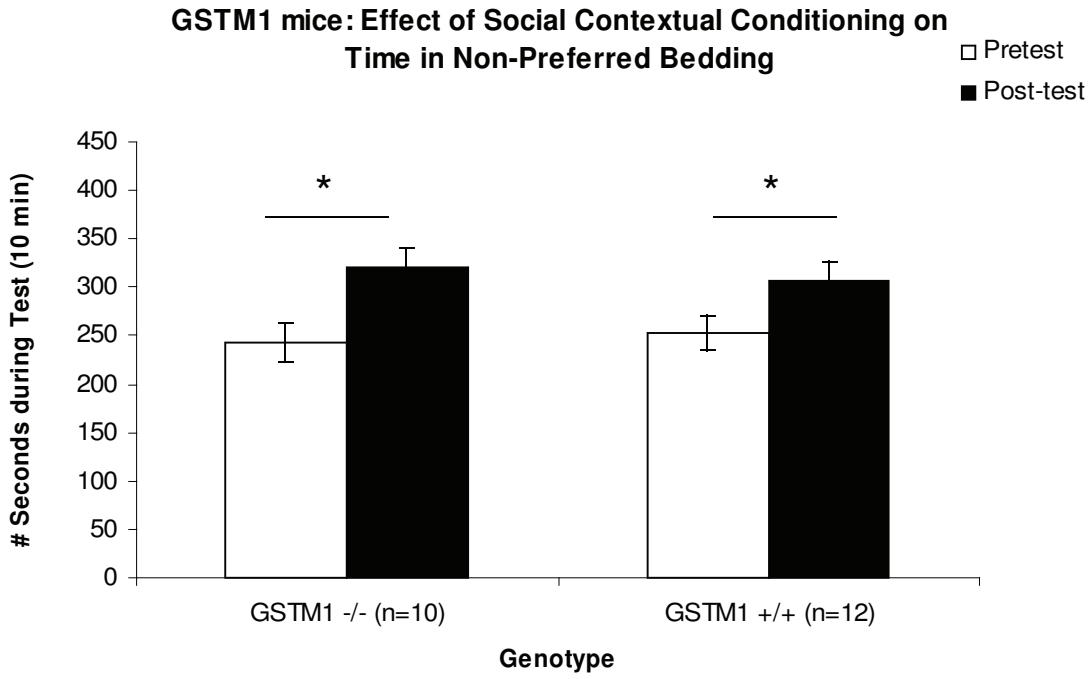


Figure 10b: Social conditioning in GSTM1 (chance level responding)

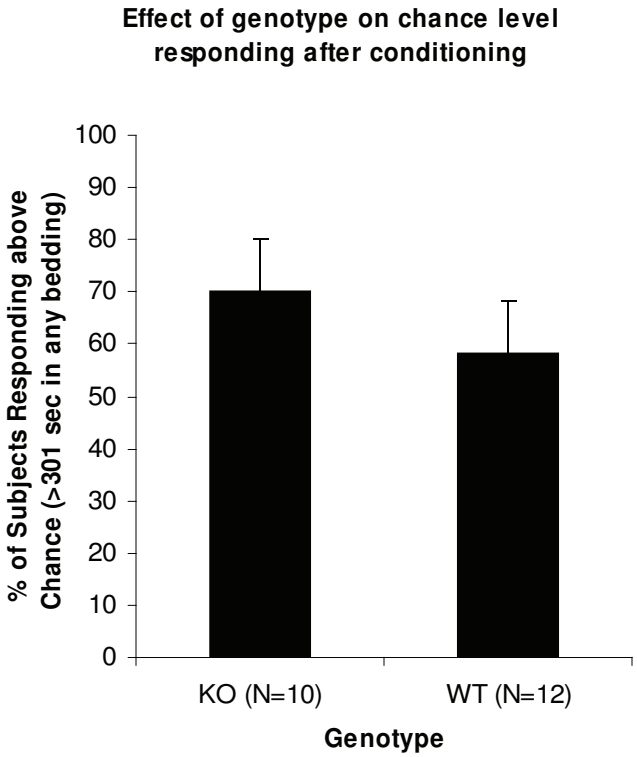


Figure 11: Social conditioning in GSTM1 (crossovers between beddings)

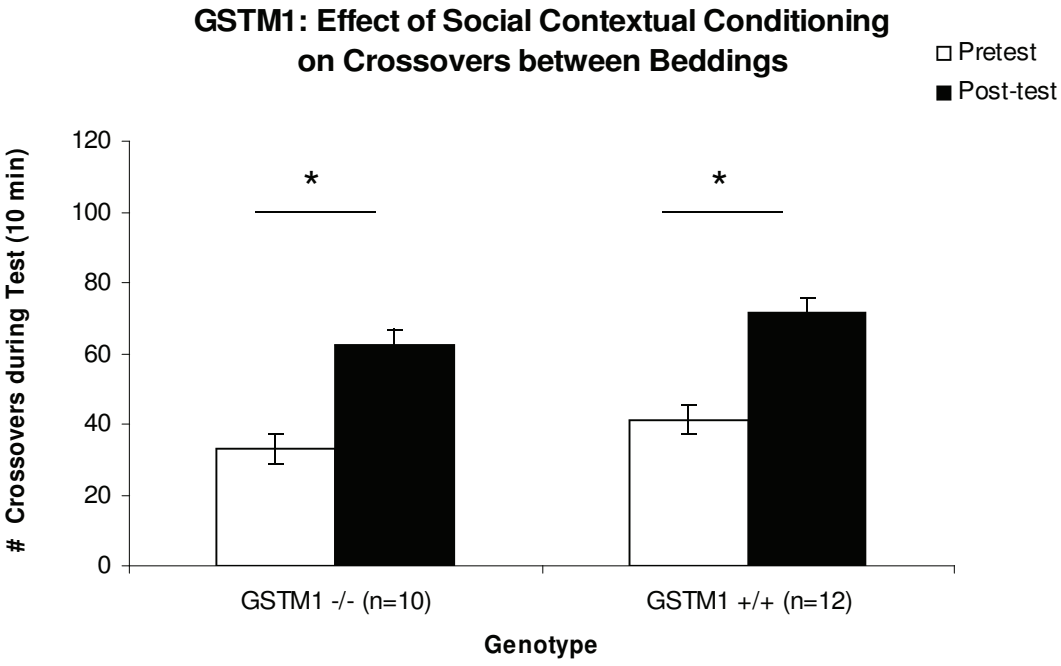


Figure 12: Food conditioning in GSTM1 (time in bedding)

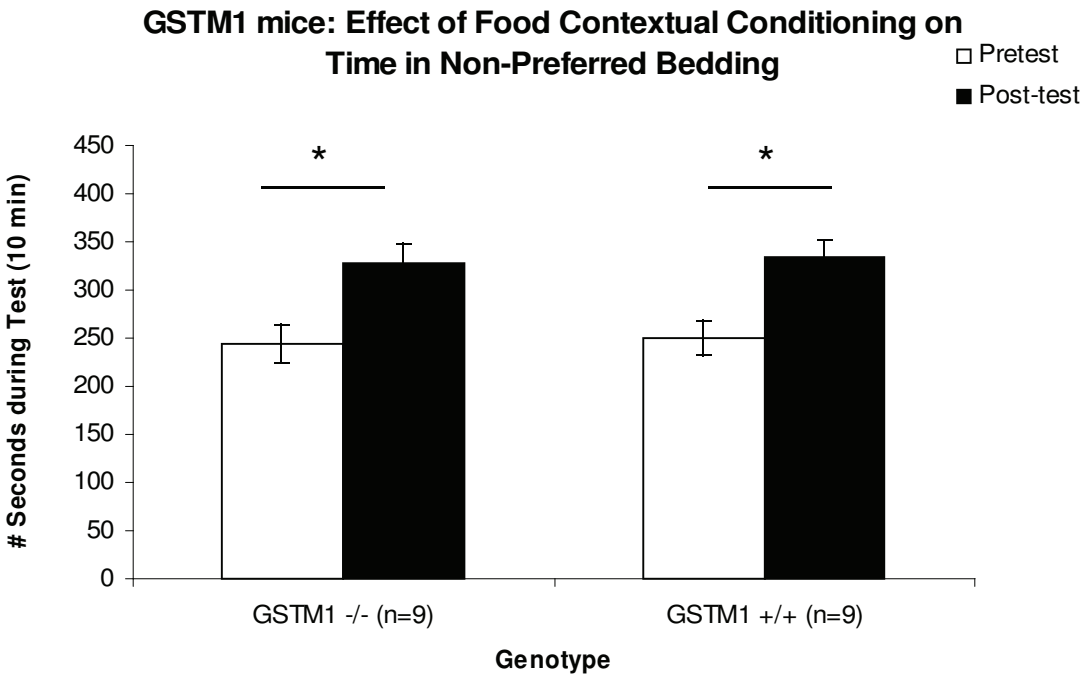


Figure 13: Food conditioning in GSTM1 (crossovers between beddings)

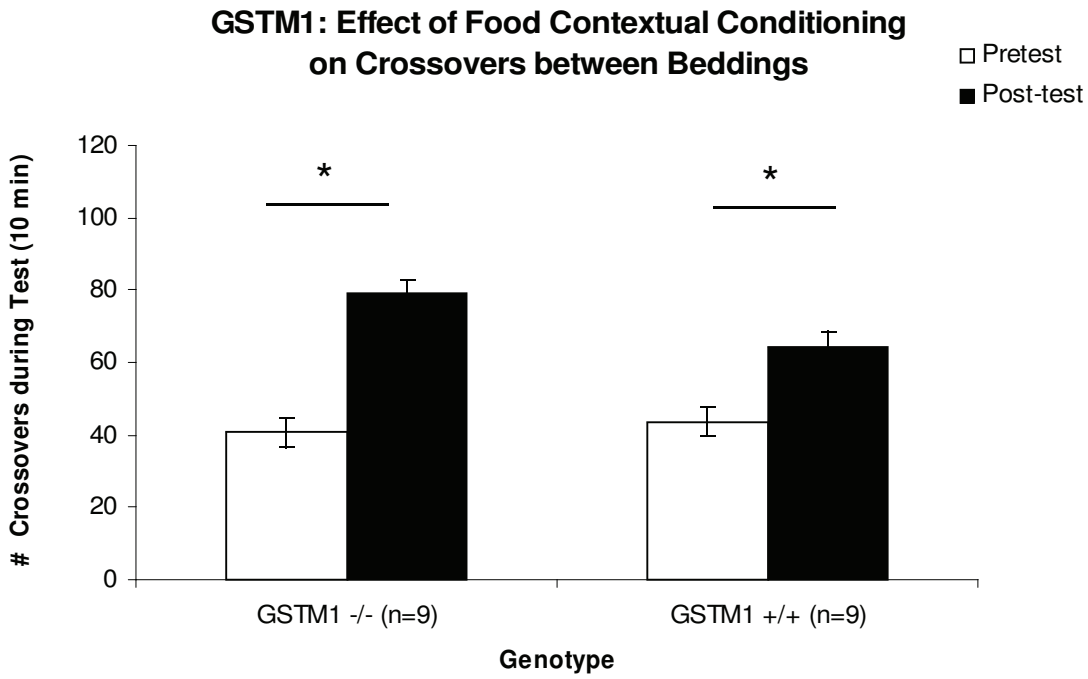


Figure 14: VPA treatment in BALB/c (time in bedding)

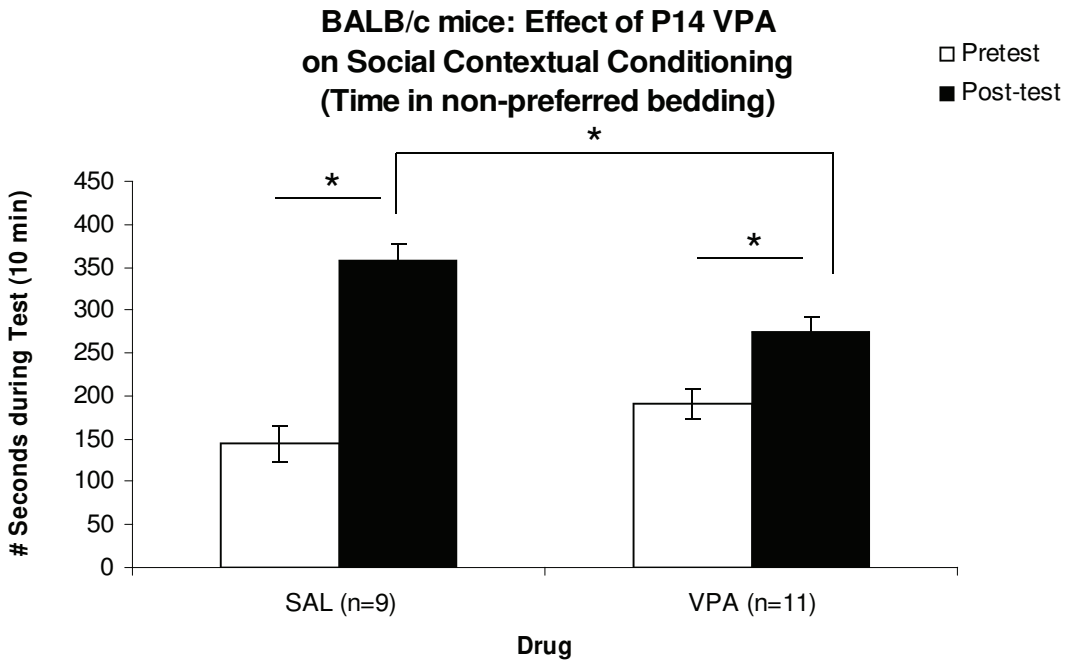


Figure 14b: VPA treatment in BALB/c (chance level responding)

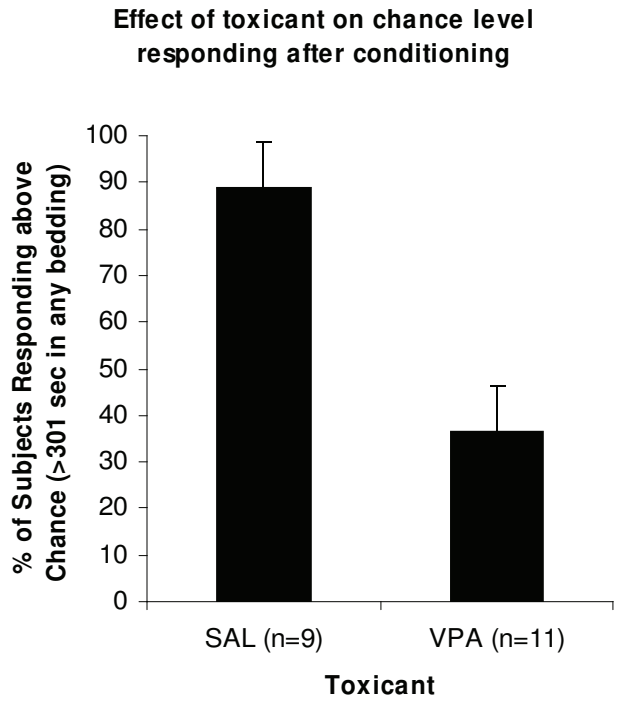


Figure 15: VPA treatment in BALB/c (crossovers between beddings)

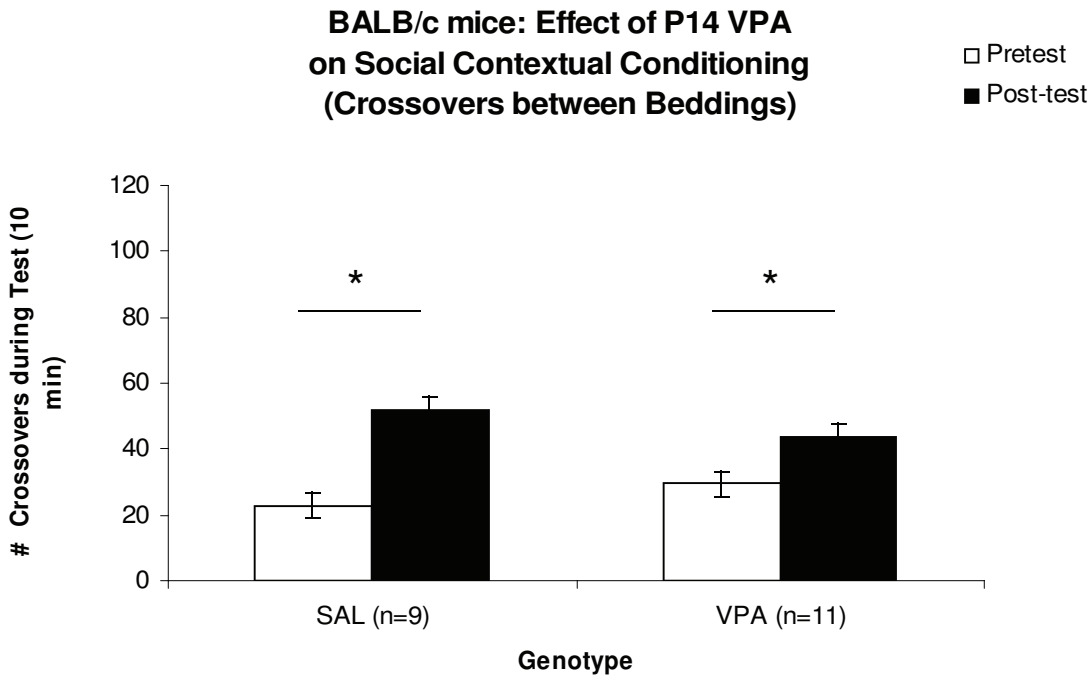
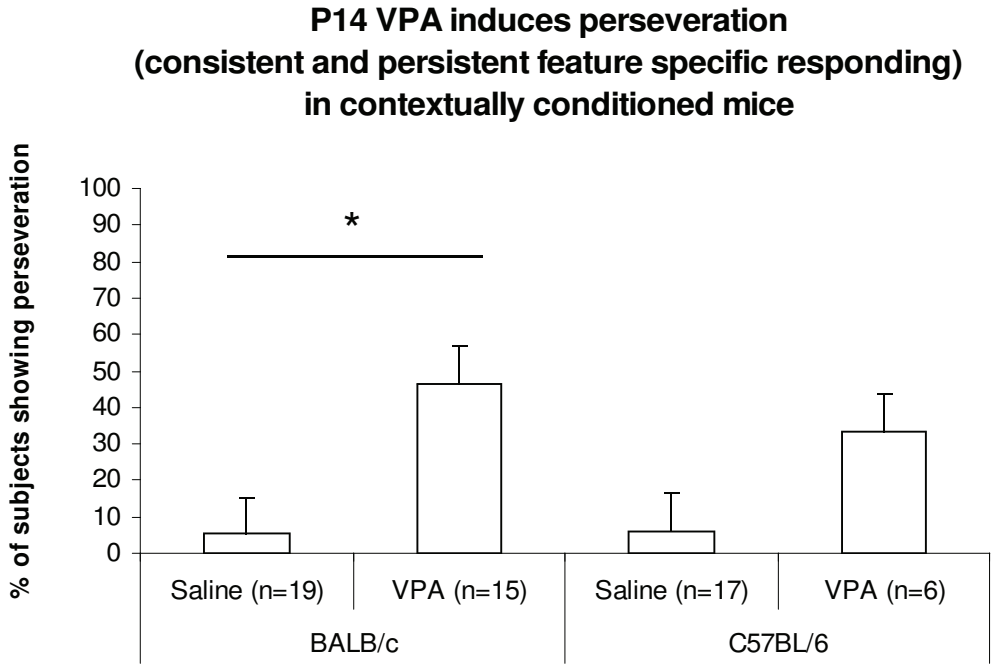


Figure 16: VPA treatment in BALB/c and C57 (perseveration)



Curriculum Vitae

Teresa Camille Parsons

Dates	Academic History
2008-2010	Degree: Doctor of Philosophy, Rutgers University Major: Behavioral Neuroscience (Psychology Department)
2005-2008	Degree: Master's of Science, Rutgers University Major: Behavioral Neuroscience (Psychology Department)
2001-2005	Degree: Master's of Science, University of North Texas Major: Behavior Analysis (Behavior Analysis Department)
1997-2001	Degree: Bachelor's of Science, University of North Texas Major: Behavior Analysis (Behavior Analysis Department)

Dates	Employment History
2005-2010	Graduate Student and Teaching Assistant, Psychology Department, Rutgers University
2009-2010	Instructor, English Department, Rutgers University
2004-2005	Supervisor, Behavior Analysis Teaching Assistant/Teaching Fellow System, Behavior Analysis Department, University of North Texas
2003-2004	Supervisor, Autism Laboratory, Behavior Analysis Department, University of North Texas
2002-2004	Research Assistant, Physiological Psychology Laboratory, Behavior Analysis Department, University of North Texas
2003-2004	Instructional Design Technologist, Behavior Analysis Department, University of North Texas
2002-2004	Instructor, Behavior Analysis Department, University of North Texas
2002-2003	Data Manager, Dallas Fort Worth Center For Autism, Carrollton, Texas
2001-2002	Case Manager, Dallas Fort Worth Center For Autism, Carrollton, Texas

Dates Publications

2010 Parsons, T. C. et al. *Modeling social rapport in adolescent BALB/c, C57BL/6, and GSTM1 knockout mice.* Poster presentation. Four Corners Association for Behavior Analysis Conference, Park City, UT

2008 Parsons, T. C. and Otto, T. (2008) Temporary inactivation of dorsal hippocampus attenuates explicitly nonspatial, unimodal, contextual fear conditioning. *Neurobiology of Learning and Memory, 90,* 261-268.

2008 Parsons C. & Otto T. *Fear generalization accompanies hippocampal-dependent, explicitly nonspatial contextual fear conditioning.* Poster presentation. Annual meeting of Society for Neuroscience, Washington, D.C.

2007 Parsons C. & Otto T. *Temporary inactivation of dorsal hippocampus attenuates explicitly nonspatial contextual conditioning.* Poster presentation. Annual meeting of Pavlovian Society, Austin, TX

2007 Otto T., Parsons C., Czerniawski, J., & Ramamoorthi, K. *Hippocampal contributions to trace, space, and context: Dissociable roles for dorsal and ventral hippocampus.* Paper session. Annual meeting of Pavlovian Society, Austin, TX

2006 Parsons T. C. & Otto T. *Temporary inactivation of dorsal hippocampus attenuates explicitly nonspatial, unimodal, contextual conditioning.* Poster presentation. Annual meeting of Society for Neuroscience, Atlanta, GA

2006 Otto T., Czerniawski J. & Parsons C. *Assessment of conditioned fear using multiple measures.* Poster presentation. Annual meeting of Society for Neuroscience, Atlanta, GA

2003 Gudmundsdottir K., Parsons-Agster T. C., Ala'I Rosales S., Haycraft C., Jenkins J., Rosales-Ruiz J., Sawyer R., & Shively J. *A Glimpse at Training Programs in an Autism Center: From Performance Management to the Finer Components of Naturalistic Instruction.* Symposium. Annual Conference for the Association of Behavior Analysis, San Francisco, CA

2003 Ala'I Rosales S., Parsons-Agster T.C., Almon H., Palinsky K., & Sawyer R. *Meaningful Outcomes in Autism Treatment at Home and School.* Symposium. Annual Conference for the Association of Behavior Analysis, San Francisco, CA

2003 Parsons T. C., Ala'I Rosales S., Sawyer R., & Gudmundsdottir K. *Implementing a Measurement System to Assess Social and Play Behaviors of Young Children with Autism in a Center-Based Preschool.* Poster presentation. Texas Association for Behavior Analysis Regional Conference, Dallas, TX

2002 Sawyer R., Delgado V., Batson C., Palinsky K., Parsons-Agster T. C, Wheetley B., & Edwards B. *A Glimpse at Play Programs for Children with Autism in a Center-Based Preschool: Play with What? Who? Where? How?* Symposium. Annual Conference for the Association of Behavior Analysis, Toronto, CAN